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UNITED STATES NATIONAL MUSEUM
Bulletin 120

THE OPALINID CILIATE INFUSORIANS

BY

MAYNARD M. METCALF
Of the Orchard Laboratory, Oberlin, Ohio

SMITHSONIAN INSTITUTION
274464
U. S. NATIONAL MUSEUM

JUN 11 1923

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The scientific publications of the United States National Museum consist of two series, the *Proceedings* and the *Bulletins*.

The *Proceedings*, the first volume of which was issued in 1878, are intended primarily as a medium for the publication of original, and usually brief, papers based on the collections of the National Museum, presenting newly acquired facts in zoology, geology, and anthropology, including descriptions of new forms of animals, and revisions of limited groups. One or two volumes are issued annually and distributed to libraries and scientific organizations. A limited number of copies of each paper in pamphlet form, is distributed to specialists and others interested in the different subjects as soon as printed. The date of publication is printed on each paper, and these dates are also recorded in the tables of contents of the volumes.

The *Bulletins*, the first of which was issued in 1875, consist of a series of separate publications comprising chiefly monographs of large zoological groups and other general systematic treatises (occasionally in several volumes), faunal works, reports of expeditions, and catalogues of type specimens, special collections, etc. The majority of the volumes are octavos, but a quarto size has been adopted in a few instances in which large plates were regarded as indispensable.

Since 1902 a series of octavo volumes containing papers relating to the botanical collections of the Museum, and known as the *Contributions from the National Herbarium*, has been published as bulletins.

The present work forms No. 120 of the *Bulletin* series.

William deC. Ravenel,
Administrative Assistant to the Secretary,
in charge of the United States National Museum.

Washington, D. C., March 24, 1923.

FOREWORD.

Most of the material used in the study of the approximately 150 new species, subspecies, and *formae* described were obtained from museum specimens of Anura that had lain long in alcohol, some for more than 80 years. While much of this material was in remarkably good condition, allowing the study even of cytological details, it is readily understood that the material as a whole was far from satisfactory, both because too limited in extent and because not always well preserved. Divergent races are prevalent in many species of Protozoa, and abundant material from different sources should be used in taxonomic studies in this group. Because of the limited material, often ill preserved, the author regards his results as only tentative, constituting a preliminary review of the taxonomy of the Opalinidae and subject to extensive modification through intensive study of more favorable material.

Similarly, in the chapter devoted to a discussion of the geographic distribution of the Anura and their Opalinid parasites, many of the suggestions are but tentative, pending a more thorough knowledge of geographic conditions in previous geologic periods. This chapter is written more for the sake of emphasizing a method of study of paleogeographic problems, the host-parasite method we may call it, than as a definitive contribution to paleogeographic knowledge. Concurrent evidence from the geographic distribution of both animals and their parasites, or host plants and their parasites, is so much more convincing than evidence from either the hosts or the parasites alone, that the host-parasite method of studying these problems can hardly be overemphasized. This method, rather than the particular suggestions in detail, is the thing to be emphasized in the chapter upon geographic distribution.

M. M. M.

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THE OPALINID CILIATE INFUSORIANS.

By Maynard M. Metcalf.
Of the Orchard Laboratory, Oberlin, Ohio.

1. INTRODUCTION.

This paper was completed for publication April 20, 1921. During the years 1906 to 1908 the author was engaged in studies of the European species of Opalinidae, investigating the structure of the known European species and following the life history of *Protoopalina* ["*Opalina*"] *intestinalis* and of *P. caudata.* Observations were also made of the sexual and presexual phases of the life-cycle in *Opalina ranarum* and *Cepedea* ["*Opalina*"] *dimidiata.* The results of these studies were published in four papers.[1] Previous to these studies the work of others, especially Engelmann (1875 and 1876), Zeller (1877), Bezzenberger (1904), and Leger and Duboscq (1904, *b*) had given us a good idea of the general character of twelve species of the family (*caudata*, *coracoidea*, *dimidiata*, *intestinalis*, *lanceolata*, *lata*, *longa*, *macronucleata*, *obtrigona*, *ranarum*, *saturnalis*, and "*zelleri*")[2], and Neresheimer (1906, 1907) had published the results of his studies of the life-histories of *Cepedea* ["*Opalina*"] *dimidiata*, and *Opalina ranarum.* Stokes (1888) "*Opalina flava*" can not be identified from his description. Additional species have since been described as follows:

Opalina virgula, by Dobell (1910),
Protoopalina ["*Opalina*"] *binucleata*, by Raff (1911).
Protoopalina ["*Opalina*"] *hylarum*, by Raff (1911).
Protoopalina ["*Opalina*"] *mitotica*, by Metcalf (1912),
Protoopalina ["*Opalina*"] *tenuis*, by Raff (1912).
Protoopalina ["*Opalina*"] *dorsalis*, by Raff (1912).
Protoopalina ["*Opalina*"] *acuta*, by Raff (1912),
Opalina cincta, by Collin (1913),
Protoopalina ["*Opalina*"] *primordialis*. by Awerinzew (1913).
Zelleriella ["*Opalina*"] *antilliensis*, by Metcalf (1914).
Zelleriella ["*Opalina*"] *brasiliensis*, by Pinto (1918), and

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[1] See the Literature List, Metcalf, 1907, *a*; 1907, *b*; 1907, *c*; 1909.
[2] This "species" is treated in the present paper as a *forma* of *Cepedea dimidiata.*

an unnamed species briefly described by Stevenson (1911), which I am naming *Protoopalina stevensoni*. As *Cepedea* ["*Opalina*"] *zelleri* is apparently not a valid species, there were 24 recognized species of Opalinidae before the present studies, one of these, *Cepedea(?)* ["*Opalina*"] *flava*, being doubtful.

Since the summer of 1908 the author has been collecting and observing living specimens of American and West Indian Opalinidae and preserved material from all parts of the world. During this period he has been much indebted to many persons for assistance and courtesies in connection with these studies. The Marine Biological Station at Naples preserved and sent intestines of the Mediterranean fish *Box boops*, containing *Protoopalina saturnalis* in fine condition. Prof. E. A. Andrews, of Johns Hopkins University, sent me important material (adult *Bufo marinus* and its tadpoles, from Jamaica, and tadpoles from an undetermined Anuran from Bromelia leaf cups in Jamaica). H. H. and C. S. Brimley, of Raleigh, North Carolina, collected for me Anura and Urodela. Dr. H. C. Fortner, of the University of Tennessee, sent me Opalinas from Michigan. Prof. E. L. Mark, director of the Bermuda Biological Laboratory, has obtained for me, through his pupil, Mr. C. S. Simkins, important data as to the breeding habits of *Bufo marinus* in Bermuda, and Dr. Leonhard Stejneger has repeatedly given me assistance in matters of taxonomy of the Amphibia, reviewing my list of names of the forms studied, bringing the nomenclature into harmony with that adopted at the United States National Museum. He has also given considerable zoogeographic data. Professor Hegner kindly sent me an excellent slide of a form collected by Dr. C. E. Simon, which, by permission, I am describing under the tentative name *Opalina* [*larvarum*]. With this slide was sent a manuscript copy of a paper (Hegner and Hsiang-Fong Wu, 1921) upon this *Opalina*. I wish very cordially to thank these authors and Doctor Simon.

Several institutions have generously extended to me their hospitality during periods of work ranging from two weeks to eight months. For this kindness I wish to express my most cordial thanks to the Scripps Institution of Biology and its director, Prof. William E. Ritter; to the University of California and the director of its zoological department, Prof. C. A. Kofoid; to the University of Washington, and to Prof. Trevor Kincaid, director of the Puget Sound Biological Laboratory; to the authorities of the United States National Museum, and especially to Dr. Leonhard Stejneger, curator of vertebrates in this museum; and to Johns Hopkins University and members of the faculty of its department of zoology, Profs. H. S. Jennings, E. A. Andrews, and S. O. Mast.

After this paper was mostly written the American Museum of Natural History, in New York City, most kindly offered, through its director, Dr. F. A. Lucas, and its curator of reptiles and amphibia, Mary C. Dickerson, to send me material of *Pipa* from South America and of *Xenopus* from Africa. This material was especially welcome because the United States National Museum specimens of these genera had proven to be uninfected and the parasites of these members of the family Pipidae are of peculiar interest, first, because it is the most archaic family of the Anura, and second, because of the geographical distribution of the family, the *Pipinae* living in northeastern South America and the *Xenopodinae* in Africa. I wish to express most cordially my thanks for this material.

The major interest in this paper is associated with data from the material gathered from the collections of the United States National Museum; and I can not adequately express my thanks to the authorities of the museum, and especially to Doctor Stejneger, for the privilege of taking Opalinids from the Anura in their collections and for the facilities they gave me for the work for three months during the winter of 1916–17. Such liberal conception of the use of museum facilities, and especially museum material, is far from universal. The author sincerely hopes that his fortunate finds will be held to justify this liberality. Should any other museums with extensive material undertake to have this searched for intestinal parasites, the author will gladly send them for comparison specimens of the species described in this paper, so far as his material will allow.[3]

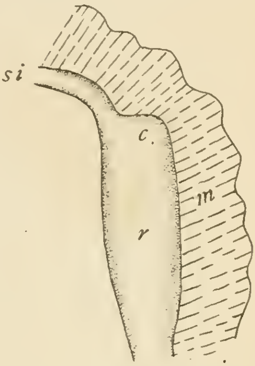

FIG. 1.—SIDE VIEW OF RECTUM OF FROG: *c*, RUDIMENTARY CAECUM; *m*, DORSAL MESENTERY; *r*, RECTUM; *s, i*, SMALL INTESTINE.

Opalinids live in the rudimentary caecal portion of the rectum of their Anuran[4] hosts (fig. 1). When they are exceedingly numerous they may be found in the whole upper portion of the rectum. Only in a few instances have I found Opalinids in the small intestine of the Anuran host, and in several of these cases the host was evidently in abnormal condition, the intestinal tract showing inflammation. In studying material from living hosts, it is easiest to remove the whole rectum to a small dish ("Syracuse solid watch glass") and, opening carefully the caecal region, to collect with a pipette, or as a cover-glass smear, material for study. Cover-glass smears killed at once, without drying, in hot corrosive sublimate-acetic acid solution, in hot corrosive sublimate solution, or in Schaudinn's corrosive sublimate-absolute alcohol solution, give very satisfactory results. Delafield's hematoxylin, overstaining and reducing, is the most easily and accurately controllable stain and gives on the whole the best results. Borax-carmine, alum-carmine, Lyons blue, and many other stains are satisfactory. With very thin species, and for the study of the cytoplasmic spherules in any species, iron hematoxylin gives very clear results. The Opalinidae present no special difficulties in preserving or staining, except that stains which color the cytoplasmic spherules obscure the nuclei in total preparations, except of the flattest species. For microscopic sections, the use of paraffine imbedding with gelatine capsules and Lefevre watch glasses is very satisfactory.[5]

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[3] See Section 10, p. 448, in which is given a list of the museums and laboratories in which the author is depositing series of paratype slides.

[4] In the much rarer infections of Urodeles they appear throughout the posterior third or half of the intestines, and the same is true of *Protoopalina saturnalis* in the marine fish *Box boops*.

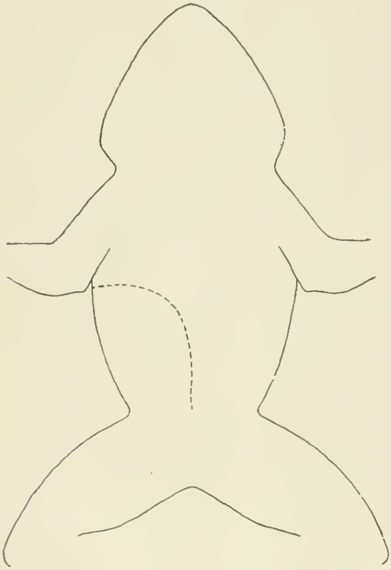

Fig. 2.—Ventral surface of a frog. The dotted line indicates the form of cut through the abdominal wall to give access to the anterior end of the rectum.

In collecting Opalinidae from frogs and toads preserved in alcohol or formalin no real injury need be done to the host. The rectum is either axial or turned to the right side. The upper end of the rectum with the rudimentary caecum in many species lies well up toward the right arm. I have found a boomerang-shaped cut on the ventral surface most convenient (fig. 2). Care, of course, should be taken, with museum specimens, not to cut the posterior cartilage of the sternum. The triangular flap of abdominal wall, released by the incision, may be bent back, and there is abundance of room for exploring the abdominal cavity and locating the rectum. When found, this can be pulled out through the incision and its upper portion slit open. The caecum is dorsal (fig. 1), so it is well to slit the rectum on one side. I have found a narrow section lifter, 3 mm. wide, the most convenient tool for taking out the rectal contents.

The ventral abdominal incision does really no injury to the host as a museum specimen. Indeed careful collectors usually open the ventral abdominal wall before placing a frog or toad in the preserving fluid. In the 1,079 operations made upon the specimens in the National Museum I injured externally but 2, and these only very slightly, by tearing the incision open farther than intended. The injury to the gut, however, is greater. Its folds are often disarranged. In many of the smaller forms the rectal wall is delicate and after it is slit open and the contents removed it collapses and study of the natural structure of the upper part of the rectum would afterwards be difficult in these operated specimens. In about 20 instances out of the 1,079 operations, the rectum broke at its junction with the small intestine. These injuries, external and internal, interfere not at all with the usual use of museum specimens and they are on the whole so slight that they should hardly cause one to hesitate to study museum material of species of Anura whose rectal parasites are insufficiently known. Many museums have much material very valuable for such study. We have no observations upon the Opalinid parasites of *Dyscophinae*, *Dendrophryniscinae*, *Amphignathodontinae*, *Hemiphractinae*, *Genophryninae*, or *Ceratobatrahinae*, and there are many genera of other families whose Opalinid parasites are unknown. Section 8 of this paper gives a list of all genera and species from which, so far as the author can learn, Opalinidae have been reported. In this list only the starred species indicate infection. Any genus or species not in this list, or in the list and not starred, is not known to the author to harbor Opalinidae.

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[5] See Metcalf (1909), p. 9.

In addition to the Opalinidae, the National Museum material gave many infections of rectal Nematodes and nearly as many of *Nyctotherus* and *Balantidium*, also some *Discophrya* and some Trematodes. These have been or will be given to special students of these forms for study.

It is important to note that alcoholic specimens of Anura have the parasitic Opalinidae far better preserved than do formalin specimens. The latter seldom yield any Opalinids, though in specimens which had the abdominal wall opened before preservation Opalinids may occasionally be found, and rarely specimens not so opened may yield Opalinids. A large percentage of the failures in my exploration of National Museum Anura was undoubtedly due to formalin preservation. Alcoholic material, on the other hand, if well preserved, shows the Opalinids, if present, in good condition. Oftentimes nuclear structure may be studied and the chromosomes counted. Even in very soft specimens of the Anura, to one's surprise, he often finds the Opalinids well enough preserved for taxonomic study. Fifty-five per cent alcohol, the strength usually used for Anura, gives thoroughly satisfactory preservation of the Opalinidae, and it is evident that Opalinids so preserved maintain their form and remain intact when the alcohol in which they and the host are preserved weakens afterwards sufficiently to allow the tissues of the host to become very flabby.

A word of explanation seems due in view of the absence of a general summary of the conclusions reached in this paper. Section 2 is itself a summary of the structure and life history of a sample species; section 3 deals with detailed taxonomy and can not well be summarized beyond the final classificatory table; sections 5 and 6 are themselves in a measure summaries of the data in the earlier parts of the paper; section 7 contains summaries of the indications found in the different phases of its discussions; and section 8 summarizes under each Anuran family the infection data contained in its long table. The nature of this paper is such that it is little better adapted to summarizing than would be an encyclopedia. The table of contents shows the nature of each section of the paper, and the index will guide one promptly to the data and discussions upon any of its points. The author is unable to provide a brief statement of the data, indications, and conclusions, perusal of which might serve as a substitute for reading the paper.

2. THE STRUCTURE AND THE LIFE HISTORY OF PROTOOPALINA INTESTINALIS.

The significance of much of the data in this paper might be obscure to one who had not in mind the chief phenomena of the structure and life cycle in the Opalinidae. For this reason it seems important to give here an outline of both the structure and the life history of a sample species. This seems the more worth while since no accurate succinct account has been published. Zeller does not describe the sexual phases of the life cycle and is misled as to the encysted phase, and Neresheimer's account includes some statements which later studies have failed to confirm. My own account of the life history, based on the study chiefly of two binucleated species, omitted certain phenomena described by Neresheimer and interpreted by him as presexual degeneration of the nuclei and the formation of secondary generative nuclei from chromidia. In some of the species observed in the present study I think I have some of the phenomena observed by Neresheimer, but their interpretation is still doubtful. Neresheimer's and the author's previous accounts are too detailed for the most convenient use by those interested in the general course of events rather than the minutiae.

Protoopalina intestinalis, the first discovered of the binucleated Opalinidae, has been reported as parasitic in *Bombina bombina* ["*Bombinator igneus*"], *Bombina pachypa* ["*Bombinator pachypus*"], *Discoglossus pictus*, *Pelobates fuscus*, *P. cultripes*, *Bufo cala-*

mita, *B. mauritanicus*, *Rana esculenta* and *Triturus vulgaris* ["*Triton taeniatus*"]. Its general appearance is shown in figure 3. It is a species of rather large size, being generally from 0.18 mm. to 0.36 mm. in length during the major part of the year, while living in the rectum of its adult host. The broadly rounded anterior end is usually turned to one side. The posterior end of the body tapers to a rounded point.

The whole surface of the body bears cilia which are arranged in longitudinal, somewhat spiral rows. In this species, as in most others, the main rows of cilia run the whole length of the body, but, anteriorly, accessory rows are interpolated between the main rows (see fig. 163, *e*, p. 194), causing the cilia to be more numerous to the unit of width upon and near the front end of the body. In *Protoopalina intestinalis* the rows of cilia over the anterior quarter of the body are about twice as close together as they are over the posterior three-quarters. In some other species the accessory rows of cilia, interpolated anteriorly, are still more numerous, the cilia rows in front being three times as close together as they are behind, or in a few species four times. In some species, on the other hand, the cilia rows are almost as closely placed behind as in front.

All the cilia in *Protoopalina intestinalis* are alike in form and size. In none of the Opalinidae known are the cilia upon the body of different kinds, except that in several species the cilia grow gradually shorter toward the posterior end of the body, and in a few species the posterior end of the body is naked. The axial fiber of

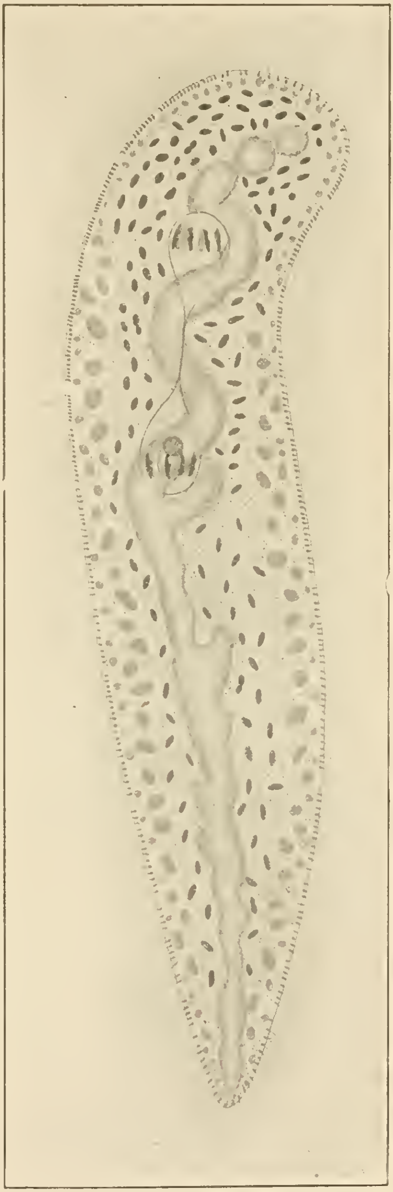

FIG. 3. — PROTOOPALINA INTESTINALIS: SEMI-SCHEMATIC, LONGITUDINAL, OPTICAL SECTION, × ABOUT 460 DIAMETERS.

each cilium arises from a spherical[6] basal granule which lies just beneath the pellicle.

The pellicle is a fairly firm membrane of visible thickness. To its tension is probably due the shape of the body. The pellicle bears longitudinal and slightly spiral grooves which are demonstrated only with difficulty. In these grooves are set the chief rows of cilia. If the accessory rows of cilia are set in grooves at all, these seem to be less developed than those which bear the main rows of cilia.

Immediately beneath the pellicle is an outer layer of ectosarc which bears no clearly visible alveoles, but seems finely granular. In figure 3 it is shown unstippled. It is in this granular layer that the basal granules of the cilia lie. The basal granules in each longitudinal row are united by an extremely delicate longitudinal thread. Similarly the basal granules of adjacent rows seem to be connected transversely by very delicate fibrillae, though the appearance is more vague and less sharply defined. In this way is formed a network of delicate fibrillae with squarish meshes and bearing at each (?) node a basal granule of a cilium. This fibrillar network probably serves the nervous function of coördination of the movements of the cilia and may be regarded as a rudimentary nervous system comparable to the much more highly developed nervous system of many Ciliates and Flagellates, so finely described by Kofoid and his pupils. No nervous centers have been observed in connection with this network in any species of Opalinid.

Internal to the granular layer of ectosarc lies a very much thicker layer which shows numerous alveoles[7] of various sizes. It was from the ectosarc of *Opalina ranarum* that Bütschli first got his conception of alveolar structure of protoplasm. Within the larger alveoles are bodies of material, of considerable size, often roughly globular in form, which I have previously called ectosarc spherules. In figure 3 these are rather darkly stippled. Their chemical nature is undetermined. Their connection with the nutritive function, while probable, is not demonstrated. Throughout the ectosarc, in the films between the alveoles, are numerous granules, cytomicrosomes, apparently exactly similar to those in the endosarc. No cytomicrosomes are shown in figure 3.

Internal to the ectosarc lies the endosarc, occupying the axial region of the body. It is alveolated, but usually with alveoles much smaller than those of the ectosarc. Many of the endosarc alveoles contain what I have called endosarc spherules (black in figure 3), generally a good deal smaller than the ectosarc spherules and of a very different nature from the latter, as is indicated both by their structure and by their reaction to stains. In *Protoopalina intestinals* they may be ellipsoidal, or dumbbell-shaped. Heavily stained with iron-hematoxylin and strongly decolorized, the endosarc spherules show a definite structure, appearing reticular, but probably indicating that they are themselves alveolated. The nature and function of the endosarc spherules is not known. It has been suggested that they are nutritive plastids[8] similar to some of the paramylon bodies in plants. Throughout the stroma of the endosarc there are numerous granules, cytomicrosomes, apparently like those in the ectosarc.

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[6] Bezzenberger (1904) described basal granules elongated perpendicularly to the pellicle in *Cepedea longa*. In my specimens of what seems to be *C. longa* I find spherical basal granules.

[7] I am not attempting to use this word in Bütschli's sense. Indeed I am not certain to just what structures Bütschli would apply this term.

Along the axis of the body, in the endosarc, lies the more or less well developed excretory organ in the form of an elongated vacuole, or often an elongated group of contiguous vacuoles.[9] The excretory pore is posterior, lying just to one side of the posterior tip of the body. The pore is not a permanent aperture, but opens only for the occasional extrusion of excreta. Frequently, however, one finds a slight depression in the surface of the body, indicating the position of the excretory pore. Immediately anterior to the pore the excretory canal usually widens to form a chamber (fig. 28, *a* and *b*, page 53). In front of this chamber the canal may sometimes be branched, but more generally it runs forward as a single irregular tube to the level of the posterior of the two nuclei. The canal, when in its full development, invariably is in close relation to the two nuclei, either being coiled spirally around them, or being branched at this level and the two branches being spirally bent around the nuclei. In some individuals the excretory canal extends beyond the nuclei, well toward the anterior end of the body; in others it extends but little, if any, in front of the anterior nucleus.

The excretory canal is not as definite a structure as this description might seem to indicate. In most individuals it is present, but in some it is not seen. When present, it may be developed only posteriorly. In some cases it is an irregular canal posteriorly, while farther forward it appears as an irregular line of vacuoles contiguous but seemingly not fused. It seems that the excretory canals or excretory vacuoles are formed at times and disappear at other times. The structure seems to be developed at different times to different degrees according to the physiological state of the animal. Even when largest and most fully developed it has no considerable delimiting membrane, such as Schubotz (1908) found in *Pycnothrix*, its wall seeming to be little, if any, more emphasized than that of any vacuole or than an ordinary alveolar film. The excretory canal seems to be little more than a more or less transient fusion of alveoles along the axis of the body. It is usually well developed and large in individuals that have been kept for a considerable time in salt solution or Locke's solution outside the body of the host. It is probably as simple in structure as any excretory organ known among the Ciliata.

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[8] See Metcalf (1909), p. 22, also p. 89.

[9] See Metcalf (1907 *b* and *c*).

Within the posterior enlargement, "bladder," of the excretory tube, one often sees numerous small granules exactly resembling the cytomicrosomes of the endosarc and ectosarc (fig. 28, *b*), except that the granules within the bladder stain somewhat differently. With Delafield's hematoxylin they stain a dirty, dull, dark blue instead of the clearer blue shown by the cytomicrosomes. These granules are more abundant usually in the bladders of individuals kept for a time outside the host, but they are often present in freshly taken individuals.

One often finds individuals which have the posterior cilia, behind the excretory pore, entangled in a mass of débris which drags after the animal as it swims. In material freshly taken from the rectum of the host one finds only a few individuals dragging such débris after them, but in cultures kept for some time outside the host most of the individuals will show such masses of débris upon the posterior cilia. Though I have studied many thousands of living individuals, keeping them under protracted observation, I have seen only half a dozen times or so the actual expulsion of the granular mass from the excretory pore, but in some of these cases the picture was very clear. It seems, therefore, that there is no regular pulsation of the excretory bladder, but that there is an occasional contraction with expulsion of the bladder contents through the excretory pore. The excreta seem to be somewhat sticky and so to become entangled in the posterior cilia. The extrusion of the liquid contents of the bladder one would naturally regard as probably equivalent to the extrusion of liquid from the excretory vacuole of *Paramecium* or any of the higher Ciliates. But the interpretation of the granules is more doubtful. They seem to be cytomicrosomes which are cast off, but there seem to be no data to help us judge whether the casting off of these cytomicrosomes is a part of the true process of excretion. The rôle that they play does not seem to be indicated. This association of cytomicrosome granules with an excretory vacuole is not a unique phenomenon. In *Amoeba proteus* there is a concentration of cytomicrosomes in the mass of protoplasm which carries the excretory vacuole (*See* Metcalf (1910)), but only in the Opalinidae, so far as I know, do such granules find their way into the interior of the vacuole and then pass out of the body when the excreta are expelled.

Well forward in the axis of the body are usually two nuclei (always two, except in transient conditions associated with fission, and except in the presexual and sexual period). In this species the nuclei are usually somewhat pear-shaped and their more pointed ends, directed toward each other, are, in a majority of individuals, connected by a delicate thread which is merely an attenuated thread of nuclear membrane, remaining from a preceding nuclear division not as yet quite carried to the point of complete separation of the two daughter nuclei. This connecting thread is very tough. In a number of instances I have had under observation living individuals which had been broken in such a way that one of the two nuclei was firmly held within one body fragment while the other nucleus lay outside in the water, still connected, however, by the thread with its sister nucleus. Intermittent pressure upon the cover glass, under these conditions, may set up violent currents in the liquid, causing the external nucleus to whip about violently, but in no such case have I succeeded in breaking or even stretching the thread. It is strong and inelastic. From this, one can infer that the nuclear membrane as a whole is tough and inelastic.

Fission is frequent in *Protoopalina intestinalis*, as it is in other Opalinidae. We therefore find the nuclei in different conditions in different individuals, according to the mitotic stage in which we observe them. As the condition of the nuclei in the several species is emphasized in the taxonomic portion of this paper, and as the series of these conditions is of great significance, it is important to review in detail the mitosis in this introductory description. It is convenient first to describe the reticulate nucleus, corresponding to the so-called "resting nucleus"[10] and then to describe mitosis.

The reticulate nucleus (fig. 4, *a*).—The caryotheca is of appreciable thickness and is tough and inelastic. Due probably to the tension of its membrane, the nucleus may often be ellipsoidal or more or less pointed at one end or at both ends. But in the reticulate stage the nuclei are sometimes approximately spherical. The nuclear membrane is persistent in all phases of the nuclear behavior, not disappearing even during mitosis. Within the caryotheca there is, of course, both achromatic and chromatic material. The former shows numerous granules and fibrillae or films. The chromatin is in the form of (1) large flat chromatin masses interconnected by branching chromatin threads, and (2) granules somewhat larger than the achromatic granules, and themselves connected by chromatin threads. The reticulate nucleus gives the appearance of an abundant reticulum, both chromatic and achromatic. The large chromatin masses and the branching threads connecting them are superficial, lying just beneath the caryotheca. The chromatin granules and the chromatin threads connecting them lie somewhat more internal. Through the whole nucleus lie the achromatic granules and threads or films, not drawn in the figures. Perhaps the achromatic threads are really films giving the appearance of threads when seen in edge view. The chromatin threads appear to be true fibrillae. Near the center of the nucleus lies a spheroidal plasmosome nucleolus, itself seen to be vacuolated, or alveolar, when properly stained (posterior nucleus of figure 3). With some of the hematoxylin stains, Delafield's for example, the nucleolus does not usually stain. Borax carmine and numerous other stains, as for instance *Lichtgrün*, bring it out well.

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[10] This is not the usual condition of the nuclei in *Protoopalina intestinalis*. The nuclei, when not actively engaged in division, are somewhat elongated and are really in a late telophase condition.

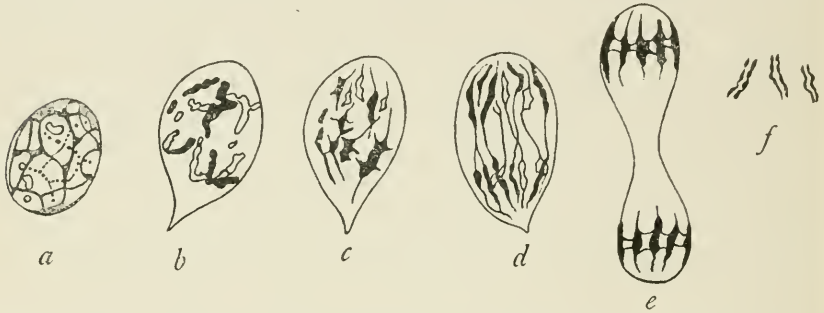

FIG. 4.—MITOSIS IN PROTOOPALINA INTESTINALIS. × 800 DIAMETERS. THE ACHROMATIC STRUCTURES IN THE NUCLEI ARE OMITTED. THE MICROCHROMATIN IS SHOWN ONLY IN FIG. *a*. *a*, A NUCLEUS IN THE RETICULATE CONDITION, ONLY THE STRUCTURES ON THE UPPER SIDE OF THE NUCLEUS ARE DRAWN; *b*, AN ANTERIOR NUCLEUS WHICH IS PASSING OUT OF THE SKEIN CONDITION, THE MACROCHROMATIN BAND BEGINNING TO DIVIDE INTO MACROCHROMOSOMES, ONLY THE MACROCHROMATIN BEING DRAWN; *c*, A NUCLEUS IN WHICH THE MACROCHROMATIN IS IN DISTINCT MASSES; *d*, AN ANAPHASE NUCLEUS; *e*, AN EARLY METAPHASE SHOWING ONLY MACROCHROMATIN IN THE DRAWING. THE MACROCHROMOSOMES ARE UNITED SIDE TO SIDE BY THIN PLATES OF CHROMATIN, DRAWN ONLY IN OUTLINE. (THIS FIGURE IS SCHEMATIC, THE INDIVIDUAL SIZE AND FORM OF THE SEVERAL CHROMOSOMES NOT BEING SHOWN.) *f*, THE APPEARANCE OF SPLITTING OF THE CHROMOSOMES IN THE STAGE IMMEDIATELY FOLLOWING THAT SHOWN IN *c*.

There is no demonstrable centrosome in either the nucleus or the cell body at any time, whether the nucleus be in "rest" or dividing by mitosis.

Mitosis seems to follow the same course in all the Protoöpalinas and Zelleriellas, and in *Cepedea* and *Opalina* the phenomena are very similar, but are not so easily followed in these smaller nuclei. In the reticulate nucleus there are, as described, a persistent caryotheca, superficial chromatin masses with their connecting branched chromatin threads, somewhat more internal chromatin granules with their branched connecting chromatin threads, and an achromatic stroma consisting of slightly smaller granules and connecting films or fibrillae. As the nucleus enters upon mitosis (fig. 4, *c*), it elongates parallel, or nearly parallel, to the long axis of the body, and one observes that the chromatin fibres of both sets[11] soon become emphasized in the direction of the elongating axis of the nucleus (see fig. 37, *f*, p. 64), while their lateral branches seem to be drawn in, at least they become fainter and less numerous, finally almost or wholly disappearing, though their disappearance is in a later mitotic stage, just as the equatorial plate stage is passing into the anaphase. The chromatin masses move toward the equator of the nucleus. The number of the chromatin masses may have been either more or less than eight in the reticulate nucleus according to the degree of concentration of the chromatin into these subcaryothecal chromatin placques. There is no clear equatorial plate but, as the period corresponding to this stage approaches, some of these chromatin masses may fuse, others may divide. One or more of them may delay dividing until the others have passed through the "equatorial plate" and have begun migrating toward the poles of the nucleus. This renders it difficult to count the chromosomes in the "equatorial plate" stage. The chromatin masses then divide, after becoming eight in number, apparently pinching apart transversely, and the daughter masses migrate slowly toward the poles of the nucleus. This migration is evidently deliberate, for one finds a goodly number of the dividing nuclei in the anaphase condition (fig. 4, *d*, *e*). Just before the daughter chromatin masses reach the poles of the nucleus they may divide, often into unequal moieties. The favorable time, therefore, for counting the chromosomes is in the middle anaphase period (fig. 4, *d*), when all the daughter chromosomes, even the laggards, have left the equator and before they have begun their polar division. Many dozens of counts of nuclei in this favorable stage have shown the chromatin masses in each daughter group to be eight in number, in the species we are describing. In the telophase (fig 4, *e*) the chromatin masses lying near the pole of the nucleus send out laterally wide bands of chromatin (shown only in outline in the figure) and thus each one fuses with its two neighboring daughter masses. In this way an irregular ring of chromatin is formed, with irregular protrusions corresponding to the ends of the formerly distinct chromatin masses. Some of the chromatin masses may already have divided and the fragments so formed may or may not be fused into the common ring-shaped group. In *Protoopalina saturnalis* (fig. 37, *a*, p. 64) the polar chromatin ring is less irregular than in *P. intestinalis*. Later the massive chromatin becomes broken up into more or less numerous bodies of larger or smaller size and by the time the two daughter nuclei have completely separated they may have assumed the reticulate condition. But the daughter nuclei are very slow in completing this process. They remain for a long time, evidently usually for days, probably for many days, in a sort of metaphase condition, the two daughter nuclei being still connected by a long thread, and the reticulate condition of the chromatin being not yet fully assumed. This is the typical condition for this species.

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[11] Those connected with the large, flat chromatin masses and with the chromatin granules.

Mention of a skein stage (fig 4, *b*) preceding the equatorial plate stage was omitted. It is often found and is sometimes very clear, but after prolonged study of thousands of dividing nuclei of this and other species I have the impression that a definite skein is sometimes not formed and that the equatorial migration of chromatin masses and their concomitant fusion or division to form the proper number may occur directly after the reticulate condition with no clear skein condition intervening. A good many nuclei are in a condition which seems best explained by such a direct transition from reticulum to equatorial plate. To determine this point the sequence of mitotic phenomena should be observel in the living nuclei, and this has not been done. Mitosis has been repeatedly studied in the living nucleus and the number and something of the condition of the chromosomes observed, even the chromatin fibers being quite visible, but the succession of events has not been observed. The abnormal conditions outside the host, while apparently stimulating the Opalinids to enter upon division, at the same time usually prevent its completion. Either the mitotic phenomena normally proceed very gradually, or they do so as a result of the abnormal environment outside the host. At any rate the phenomena do not go forward with sufficient speed to allow observation of their sequence when the animals are pressed by the cover glass and held immovable for study with immersion objectives.

Longitudinal splitting of the chromatin masses does not seem to occur in the equatorial plate. The division there is a transverse pinching apart into two portions. The resultant daughter masses remain connected in pairs across the equator of the nucleus by means of persistent chromatin threads (see figs. 28, *d*, p. 53, 49, *b*, p. 80, and 86, *b*, p. 121). One readily sees that the daughter masses of a single pair are very closely similar. In any one anaphase group the several daughter chromatin masses differ from one another with characteristic differences of size, form, and behavior (see fig. 86, *b*, p. 121), and these constant differences are observed in comparing different nuclei in different animals. There is therefore a clear individuality of these chromatin masses which is characteristic in detail of each of the eight components of the group. But though longitudinal splitting of the chromosomes does not occur in the equatorial plate, one finds often in the telophase stages that the chromatin masses show a vague yet evident double appearance, two darker-stained lateral regions having a paler strip between them (fig. 4, *f*). The three bands thus formed in each chromatin mass lie lengthwise in the mass. I am not sure whether the appearance indicates a true double condition of the chromatin masses at this stage, or is due to the presence of an achromatic core in the axis of the chromatin mass. The latter interpretation is suggested by the fact that usually all the chromatin masses in a nucleus in this phase show this appearance of splitting when seen from one point of view. If it were true splitting, some of them would naturally be seen from such an angle as to obscure their double character. Observation of mitoses of some other protozoa shows that splitting of the chromosomes in the metaphase stages is not exceptional.

The telophase may be said to be passed when the thread between the two nuclei breaks and the nuclei become more spherical. There follows at this time an apparently brief reticulate stage, with mostly small chromatin masses scattered over the periphery of the nucleus, beneath the caryotheca, these masses being connected by delicate branching fibers of chromatin. Apparently the telophase passes into the reticulate condition by the further fragmenting of the chromatin masses and their sending out during the process numerous filose and branching pseudopodia to form the fibers of the chromatin net.

As the chromatin masses of the reticulate stage pass into the skein of the following stage, some of the fibrillae of the reticulum decrease in size and others become emphasized. This process continues until the outer chromatin spindle is formed. Apparently the lateral branches are drawn in, as filose pseudopodia might be withdrawn, and the main threads, running longitudinally, are thus increased in size. Even at the time when the outer chromatin spindle is most fully developed as a spindle, its fibers still show some branching.

In the absence of a centrosome during mitosis, as at all other times, the fibers of the outer chromatin spindle are attached to the nuclear membrane at its two poles. This seems to be a permanent attachment. When the nucleus divides at the equator, its membrane pinches down upon the persistent spindle fibers and holds them permanently fastened (see *Protoopalina mitotica*, fig. 48, *e*, p. 78). The fibers seem to be a part of the persistent chromatin complex, and the chromatin seems to be autonomous in its movements and not to be acted upon by any outside agents, such as contractile fibrillae from centrosomes, to pull the chromosomes through their quadrille.

Thus far in our description of mitosis we have referred only to the chromatin masses and their chromatin fibrillae, forming the outer chromatin reticulum and the outer spindle. There is another set of chromatin structures lying more internal than these (fig. 4, *a*. See also fig. 85, *b*, p. 120). There are chromatin granules, appearing somewhat larger than the achromatic granules of the nucleus, possibly because of their darker staining. These are also connected by fibrillae best seen during mitosis, when they form a longitudinal spindle of somewhat branching fibers lying in a layer within the outer spindle. In the reticulate nucleus, these chromatin granules are scattered, showing no discernible special arrangement. Their behavior has not yet been followed through the whole of mitosis. During the anaphase the picture is clearest (see *Protoopalina saturnalis*, fig. 37, *f*, p. 63). Then these granules are aggregated into linear groups lying along the longitudinal fibers of the inner spindle. These groups differ from one another in length and in number of component granules. *Protoopalina intestinalis* has not yet been carefully studied as to the detailed character of its granule groups, but in *Zelleriella* ["*Opalina*"] *antilliensis* there are 10 of these lines of granules, differing from one another in length and in number of component granules. *Zelleriella antilliensis* has 10 of the large chromatin masses, so that the number of the chromatin masses and that of the groups of granules are the same. Reexamination of my slides of *Protoopalina intestinalis* and *P. caudata* shows about eight linear groups of granules in the former and about six in the latter, these species showing respectively eight and six chromatin masses. My preparations of these species are, however, not now in quite good enough condition for one to say definitely that their groups of chromatin granules are respectively eight and six. These are the counts obtained, but the slides, now over fourteen years old, are not clear enough to warrant a definite statement. I have not had enough favorable material of *Zelleriella antilliensis* to allow detailed study of many nuclei and I cannot say if the groups of granules show constant and characteristic differences in the number of their component granules. I hope to obtain material for testing this point and determining the question of individuality in these granule groups.

The granule groups gather in the equator of the nucleus at the mitotic phase of the mitosis and then divide each group into two, one daughter group of each pair migrating to each pole of the nucleus. No observations have as yet been made as to longitudinal splitting of the groups of granules.

We have referred to chromatin masses and groups of granules. It seems, however, justifiable to call them massive chromosomes, or macrochromosomes, and granular chromosomes, or microchromosomes, and hereafter in this paper we will so name them.

The nucleolus does not disappear or divide during mitosis, at least it does not generally do so.[12] It passes bodily into one of the two daughter nuclei, and in the other daughter nucleus a new nucleolus is formed. As already noted, the nucleolus is not composed either wholly or in part of chromatin, but is a true plasmosome nucleolus.

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[12] I have never seen evidence of the division of a nucleolus.

During the whole mitosis the nuclear membrane remains intact. Furthermore there is no considerable shifting of the axes of the nucleus with reference to the axes of the body. The longitudinal axis of the nucleus remains constantly parallel, or nearly so, to the longitudinal axis of the body. As the chromosomes, both massive and granular, remain attached to the chromatin fibers, and as these have a persistent attachment to the two poles of the nuclear membrane, we see that there is a definiteness and persistence of orientation in these nuclei that is particularly favorable for the study of some problems, which, however, I do not wish to take up in this paper.

Mitotic division of one nucleus is accompanied by similar division of the other nucleus in the Protoöpalinas and the Zelleriellas, but usually one finds that the posterior nucleus lags very slightly behind the anterior nucleus, the difference in mitotic condition being generally just enough to be observable. (See fig. 12, *b*, p. 35, and also *Zelleriella atelopyxena*, fig. 59, *b* and *c*, p. 92, in which these relations are reversed.)

Following the nuclear mitosis there is division of the body. This may sometimes be a longitudinal division, in other cases it is transverse. As these animals have not as yet been reared successfully outside the host, we have no sufficient data for determining the relative frequency of the two sorts of division. Daughter individuals which have just come from transverse division are a bit more stocky than the usual form, but daughters which have just been formed by longitudinal division are not always observably more slender than usual. It may be that the relative frequency of the two sorts of division is not the same for all strains of a given species and this divergence in division habit, if it exists, may account in part for the divergence in average form found in different infections of the same species of Opalinid.

One point in connection with these divisions is important to note. When division occurs the division plane passes between the two pairs of daughter nuclei, so that each daughter cell so formed contains both the daughter nuclei coming from the division of one of the parent nuclei. The two daughter nuclei of a single pair are never separated into different cells. but remain together in the same daughter cell, being even connected for a long time by a thread of nuclear membrane. This point is of considerable significance in comparing the Opalinidæ with the higher Ciliata, as we shall later see.

It is well to give here an outline of the life history, as we have of the structure. of the species chosen, *Protoopalina intestinalis*, for two reasons: first. for reference in our further discussions in this paper, to give a clearer understanding of some of the phenomena and relations to be treated, and, second, for the sake of having available a succinct account of this interesting animal.

During most of the year the Opalinids in the rectum of their host continue to grow, dividing occasionally by longitudinal and transverse fission as described. When the reproductive season of the host approaches, that is, in Europe and North America in the spring, the Opalinids begin to divide with greater frequency, multiplication being more rapid than growth, so that the animals become smaller, until finally they are minute forms, often with but a single nucleus. In the latter cases evidently the division of the body has gotten ahead of the division of the nucleus, so that finally the two nuclei are separated before they have time to divide. These minute individuals, containing either one or two nuclei, then encyst (fig. 5). (In the multinucleated Cepedeas and Opalinas the encysting individuals may have from 1 to as many as 12 nuclei.) The cysts pass out with the feces of the host into the water to which the hosts have now gone for

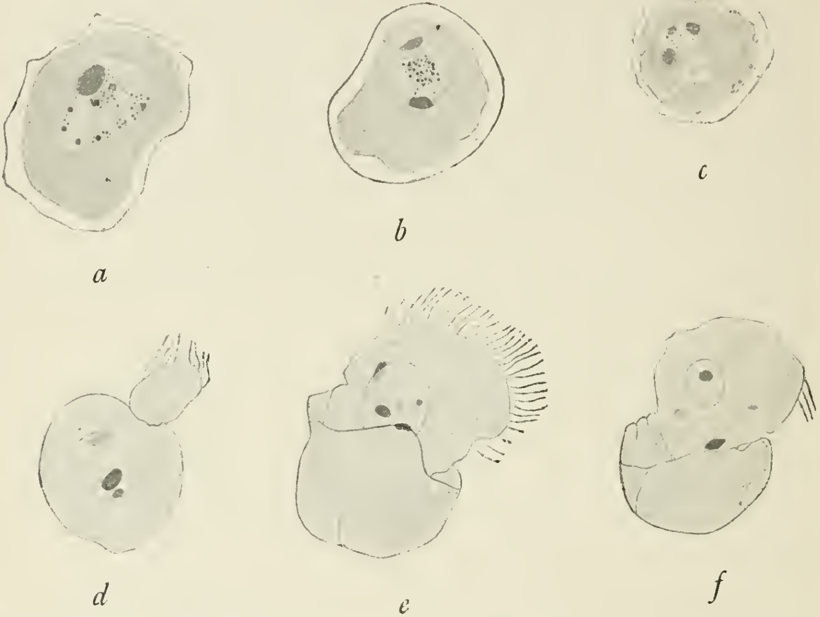

FIG. 5.—PROTOOPALINA INTESTINALIS, CYSTS: × 673 DIAMETERS. *a*, AN ENCYSTING INDIVIDUAL WHOSE NUCLEUS CONTAINS ONE LARGE MACROCHROMATIN SPHERE, 8 MUCH SMALLER MICROCHROMATIN (?) PARTICLES AND NUMEROUS ACHROMATIC GRANULES; *b*, AN ENCYSTING INDIVIDUAL WHOSE NUCLEUS CONTAINS BOTH CHROMATIC SPHERES AND ACHROMATIC GRANULES; *c*, A CYST SHOWING THREE MACROCHROMATIN SPHERES IN ITS NUCLEUS; *d*, AN INDIVIDUAL HATCHING FROM THE CYST, ITS NUCLEUS CONTAINS THREE MACROCHROMATIN SPHERES; *e*, ANOTHER HATCHING INDIVIDUAL WHOSE NUCLEUS CONTAINS FOUR (two?) MACROCHROMATIN SPHERES, A FIFTH (third?) HAVING BEEN EXTRUDED INTO THE CYTOPLASM; *f*, A SIMILAR INDIVIDUAL WHOSE NUCLEUS HAS EXTRUDED TWO MACROCHROMATIN SPHERES AND STILL RETAINS ONE. (After Metcalf, 1909.)

their own breeding. Here the cysts fall to the bottom, lying there probably for some days, until the eggs of the host species have developed into tadpoles. These tadpoles, browsing along the bottom, ingest the cysts along with their algal food.

Passing through the alimentary canal of the tadpole, the cysts, about four hours after ingestion, hatch in the rectal region (fig. 5, *d*, *e*, *f*), the little Opalinids reappearing in the same condition as that in which they entered the cyst, except that, first, they have usually extruded most of their ectosarc spherules (fig. 5, *c*), and, second, they often have extruded from their nuclei during the period of encystment some of their chromatin, in the form of from one to four rounded masses, which can be seen for a time in the cytoplasm, but soon are absorbed (fig. 5, *e* and *f*). The little Opalinids now divide several times, forming ultimately gametes of two sorts, macrogametes (fig. 7), closely resembling the ordinary forms, except that they are usually uninucleate, and microgametes (figs. 6 and 7), which are slender uninucleated individuals having a slender tapering tail devoid of cilia. The tail is bent at the base at right angles to the body, and near its naked tip is a slight swelling forming a minute ball that is sticky. The cilia of the microgamete are sparse and unusually long for so small an individual. By their rather feeble lashing the male gametes move about backwards, that is, with the pointed, posterior end leading. Upon coming in contact with a macrogamete the male attaches itself first by the sticky ball upon its tail. Then it gradually draws into the macrogamete, becoming completely fused. Its nucleus approaches and meets that of the macrogamete, both being somewhat spindle-shaped (fig. 8, *a*), and the two lying diagonally together. Soon the dividing portion of the nuclear membrane disappears (fig. 8, *b*) and the two nuclei become completely fused (fig. 8, *c*). Nere-

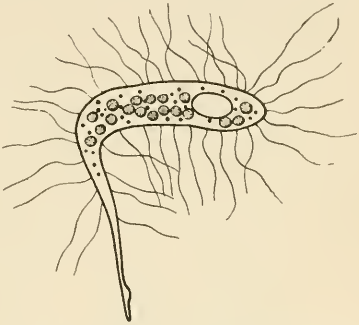

Fig. 6.—Protoopalina intestinalis; a microgamate. The ellipsoidal body, unstippled, is the nucleus. × 1,000 diameters. (After Metcalf, 1909.)

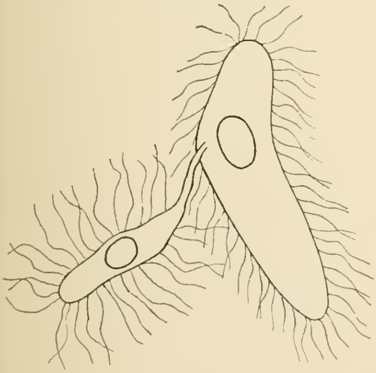

Fig. 7.—Protoopalina intestinalis; copulation of microgamete and macrogamete.

sheimer describes certain cysts with spindle-shaped nuclei found in the tadpole. These he regards as encysted zygotes. I have not observed them, nor has Brumpt (1915) been able to find them. Their further fate has not been followed. The zygotes thus formed in the recta of the tadpoles resemble the full-grown Opalinids, except for size and the fact that they have but a single nucleus. The details of development into full-sized Opalinids have not been followed. Growth and multiplication occur during the first month much as they do among the larger forms. Brumpt (1915) has found that after some weeks the Opalinids in the recta of the tadpoles show an epidemic of division followed by encystment, the cysts being infection cysts identical structurally with those in the adult hosts in the spring of the year. This process continues the abundance of the cysts in the pools and renders more certain the infection of the tadpoles which develop from the eggs that are laid late. The chief points of interest unobserved as yet are two. First is the exact manner of reestablishing the binucleated condition. As the Opalinids have not been successfully reared outside the host, and as many of the phenomena, especially those of fission, are distorted in cultures, it is not easy to learn in just what way binucleation is reestablished. It is, of course, easy to imagine, but we do not know the exact facts. The second undetermined point is the manner of reestablishing the double character of the chromatin, with a set of macrochromosomes and a distinct set of microchromosomes. This last point in question will be more clearly indicated after reading the description of the expulsion of chromatin from the nuclei at about the time of encystment. (See p. 22.)

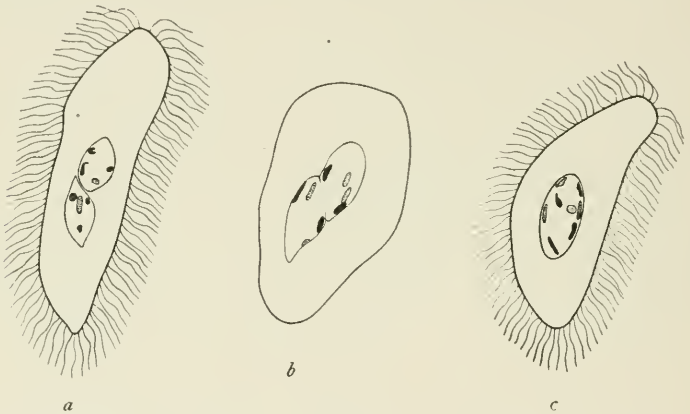

FIG. 8.—PROTOOPALINA INTESTINALIS; FORMATION OF THE COPULA NUCLEUS. × 673 DIAMETERS.

But the details of the sexual phenomena should be described. The subject needs further study, for there are certain discrepancies between Neresheimer's observations and my own and there are certain gaps which no observations cover. I had hoped to obtain ere this material for following these phenomena in detail, but circumstances beyond control have hindered my plan for obtaining the needed material in the Tropics. I will here describe the phenomena as observed, noting the gaps and discrepancies. Neresheimer described for multinucleate Opalinidae a period before the rapid spring divisions begin, during which the nuclei give off numerous chromidia into the cytoplasm and then degenerate and disappear. Meanwhile new nuclei gradually form from the extruded chromidia and these become the definitive nuclei during the further life of the animal. In my studies of *Protoopalina intestinalis* and *P. caudata* (see Metcalf, 1909) I did not find any such degeneration of nuclei, except in the case of a few individuals of *P. caudata* which were interpreted as abnormal. In recent studies of 7,000 slides of 150 species, I find in two species of binucleated Opalinids, *Zelleriella hirsuta* (new) and *Z.* [of *Bufo woodhousi*] (new), degeneration (?) phenomena (figs. 99 and 100, pp. 135 and 136, and fig. 98, p. 132) apparently related to those Neresheimer described in several multinucleated forms.[13] They

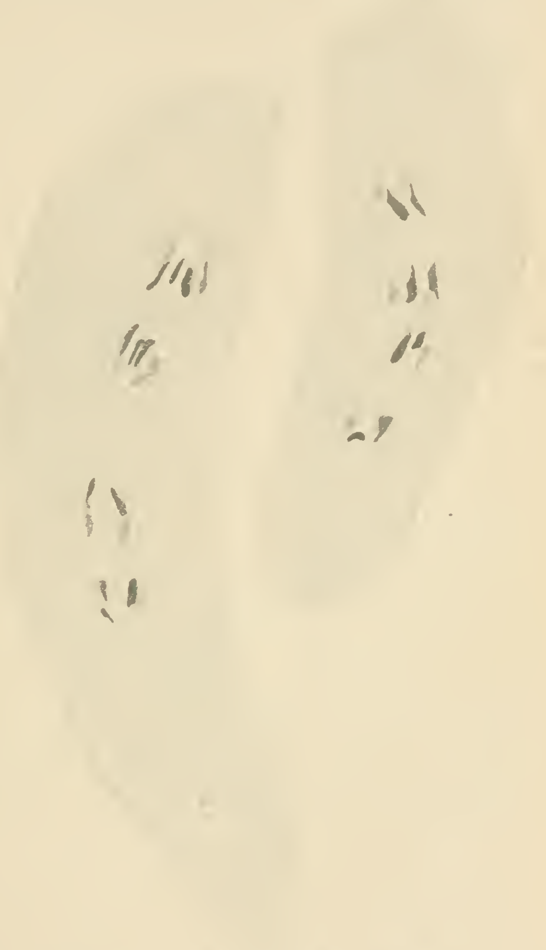

Fig. 9.—Protoopalina intestinalis; showing reduction in the number of macrochromosomes. × 673 diameters.

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[13] In a former paper (Metcalf, 1909) I described in some detail degeneration phenomena in the nuclei of *Opalina obtrigona*.

occur in each species in full-sized individuals, so they must considerably precede in time the sexual phenomena in the Opalinids in the tadpole. I am in doubt as to the interpretation of these phenomena. They seem to indicate pathological nuclear degeneration, perhaps associated with nuclear parasites, or they may possibly be normal phases of the life history, as Neresheimer described. There is need of careful restudy of the life history to determine the exact phenomena here. Figures 98, *b*, 99, *e*, and 100, *c*, show cells whose original nuclei are in active division, apparently indicating normal condition, though the cytoplasm contains numerous smaller nuclei. On the other hand, figures 99, *c* and *f*, show original nuclei which are not in normal, or at least in usual, condition, the nuclear contents having aggregated to form one or two granular spheres which somewhat resemble the small nuclei in the cytoplasm.

In *Protoopalina intestinalis* the further phenomena observed are as follows: A short time (about four generations) previous to encystment the nuclei of the already small Opalinids show a change and instead of eight massive chromosomes have but four (fig. 9). The significance of this change is not certain, but it may be synapsis which has occurred. Thereafter, until the macrochromatin is thrown away before copulation, the macrochromosome number in the nuclei is uniformly four.

During encystment, or immediately preceeding or following it, one sees in each nucleus, whether of binucleated or uninucleated individuals, from one to four, usually one to three, balls of densely staining chromatin in addition to the chromatin granules in the nucleus (fig. 5, *a*, *b*, *c*, and *d*). These chromatin balls are extruded from the nucleus into the cytoplasm and there are absorbed (fig. 5, *e* and *f*). Later, when the cysts have hatched in the tadpole, we find the small Opalinids with four chromosomes in their nuclei. The phenomena have not yet been so followed as to enable us to say whether these persistent chromosomes are formed from the chromatin granules or from the chromatin masses, though there is considerable indication that the massive chromosomes are thrown bodily out of the nucleus, having first massed together to form the chromatin balls described above. Such absorption into the cytoplasm of masses of chramatin before sexual reproduction is apparently comparable to the disintegration and absorption of the meganucleus in *Paramecium* and other Euciliates. In some of my former experiments upon *Protoopalina intestinalis* I fed small Opalinids, as well as cysts, to the tadpoles. These small Opalinids passed apparently unharmed through the intestine into the rectum of the tadpole. Then, however, they began to throw off great masses of chromatin from their nuclei into the cytoplasm (fig. 10). The pictures obtained seem to indicate the total expulsion of the massive chromosomes, leaving behind in the nucleus finally only eight small oval or spheroidal chromosomes of about the size seen in the gametes. At the time all these phenomena were observed, I did not have in mind the distinction between massive and granular chromosomes, and there is therefore need of restudy of these stages. The observations made are consistent with and would point to the conclusion that the massive chromosomes are all bodily extruded before the formation and the union of the gametes, but the conditions have not been carefully scrutinized with reference to this question, and it must therefore be regarded as still needing further study. If the massive chromosomes are wholly extruded, as seems to be the case, they must apparently be regarded as vegetative in function, like the meganucleus of Euciliates, in contrast to the reproductive granular chromosomes. If the macrochromosomes are not wholly extruded but are merely greatly reduced in bulk by extrusion of most of their substance, the comparison with the conditions in the Euciliates is even more interesting. What, then, is the meaning of the degeneration of nuclei, which has been seen at an earlier stage of the life-history in *Cepedea*, *Opalina*, and *Zelleriella*, if indeed these phenomena be not pathological? What is the relation between these two sets of phenomena? The presexual and the sexual phases

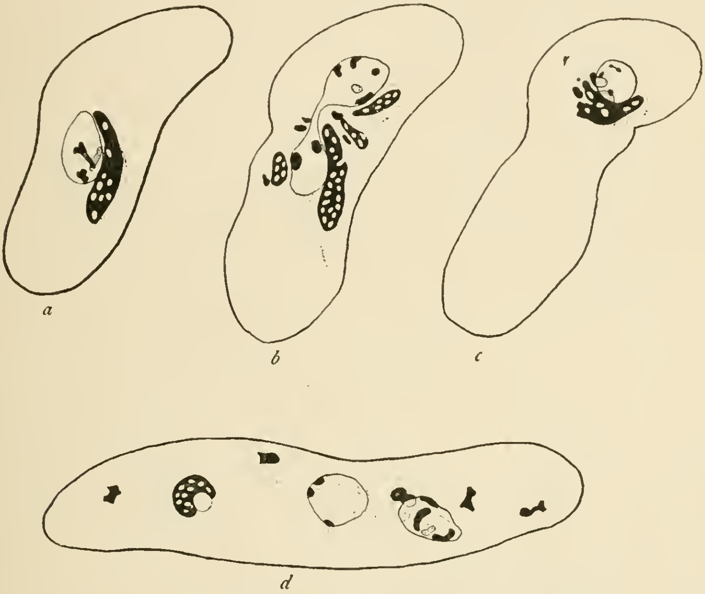

FIG. 10.—PROTOOPALINA INTESTINALIS; *a*, *b*, AND *c* ARE SERIAL SECTIONS OF ONE INDIVIDUAL; *d* IS ONE FROM A SIMILAR SERIES OF ANOTHER INDIVIDUAL. BOTH ANIMALS ARE DISCHARGING FROM THEIR NUCLEI GREAT AMOUNTS OF CHROMATIN. × 500 DIAMETERS. (After Metcalf, 1909.)

of the life-history must be followed again to determine exactly these relations.

In the definitive gametes we have uninucleated individuals, each nucleus containing four rather small chromosomes and some achromatic granules. The fusion of these nuclei gives nuclei with eight chromosomes (fig. 8, *c*). An interesting complication is introduced in the fact that fusion of the gametes may occur before the macrogamete has completed its final division and while it still has two nuclei. But for discussion of this point I would refer to a former paper (Metcalf, 1909).

It is evident from this life history, as well as from the nature of the ordinary vegetative fissions, that *Protoopalina intestinalis* is not a truly binucleated form. It is normally uninucleate at the time of sexual union, and it is uninucleate immediately after ordinary vegetative fission, before the division of the nucleus in the daughter cell has been completed, thus reestablishing the binucleate condition. The two daughter cells, in this species, usually separate while their nuclei are in a late anaphase, or often a telophase condition. These phenomena will be found significant in the discussion of speciation in the Protoöpalinas.

There is little needs here be said of the biology of the Opalinids. They seem to live as commensals with their hosts, rather than as true parasites. They apparently feed upon the wastes in the rectum, and so far as can be determined are not injurious to the animal which furnishes them their secluded and advantageous habitat. It is, of course, altogether improbable that the Opalinidae reached their present very considerable diversity before they acquired their parasitic habit. Were this the case, there would probably still be free-living forms showing undoubted resemblance to the parasitic members of the group. Their divergence must have taken place under conditions of parasitism. But the secluded habitat in the recta of their hosts, and the abundance of predigeated food, make conditions which do not emphasize the struggle for existence, and their environment does not present sufficient diversity to encourage diverse adaptations to divergent conditions of environment. Free-living Flagellates and Ciliates have structural features adapted to seeking and ingesting solid food. The Opalinids have lost all trace of structures associated with ingestion, but retain the locomotor organs. The further modifications in form and nuclear relations, which are so marked among the members of the family, were almost certainly developed during their life as parasites. Since the uniformity and favorable character of their life conditions during parasitism do not encourage diversification in structure through natural selection, it seems evident that such diversification as has occurred has been acquired through divergent orthogenesis, if we may use this Hibernianism. The causes of the divergence have apparently been internal, divergent adaptation to external surroundings having had little, if any, part in the process. We seem to have, then, in the members of this family a most instructive illustration of divergent evolution due to the inherent internal tendencies in the original stock, and to such further internal tendencies as developed during the progressive history of the group, with little control from outside influences. The environmental conditions have been remarkably uniform and constant. The variable factors in the evolution have been internal. From this viewpoint the speciation becomes particularly interesting. It will also be seen to have great interest in connection with questions of the origin of the Ciliata.

It might be claimed that the diversity between the different species of Opalinidae arose in response to diverse conditions in the chemical environment in different species of hosts, but this suggestion is rendered improbable by the conditions among the *Opalinae angustae*, a group which arose apparently among the Hylidae (a South American family) when these came into North America during the Pliocene period (see section 7) and there met the Ranas and adopted their broad *Opalinae.* In the Hylidae the broad *Opalinae* became narrow, but, as the narrow *Opalinae* spread to other species and genera of Hylidae and to species and genera of other families, they have been slow to change their character. The forms which I have had to class as *Opalina obtrigonoidea*, a new species, are found in 18 species of hosts (Hylidae 6, Bufonidae 5, Pelobotidae 1, Gastrophrynidae 1, Ranidae 5), yet their diverse hosts have not produced diversities in the parasites sufficient to be recognized as specific. This does not support the idea that the diversities in the species of Opalinidae are due largely to diversities in their chemical environment in their hosts. Factors within the Opalinidae themselves, rather than environmental conditions, seem to have been chiefly controlling in their speciation.

3. A DESCRIPTION OF THE KNOWN SPECIES AND OF THE GENERA IN THE FAMILY OPALINIDAE.

Twenty-five species of Opalinidae have been described, of which two are doubtful and a third is in this paper demoted to be merely a *forma* of another species. This paper adds 120 species (of which 18 are somewhat doubtful), 20 subspecies (of which 6 are doubtful), and 10 *formae.* Taxonomic studies in this group are difficult because of several features:

1. The several species of Opalinids for the most part seem to show racial diversities which are much more marked than, say, those of *Paramecium*, and the extreme forms of different "species" overlap. Demarcation of species in some instances seems well nigh impossible.

2. This is especially true because as yet we are not successful in rearing these animals in cultures outside the host, and comparison of pure line groups is very difficult, since it would involve infecting uncontaminated tadpoles each with a single cyst. Such infections can not be made at will, but are possible only during the period of the breeding of the host, which lasts only a few weeks and occurs but once a year. Such study seems impracticable.

3. There is considerable diversity of structure under different physiological conditions and at different stages in the life cycle. For example, in a given species—

a. The nucleus varies in size and in structural condition according to—

- α. The physiological state of the animal; and
- β. The stage of the life cycle observed.

b. The presence, degree of development, and the form of the excretory vacuole varies with the physiological condition..

c. The size of the animals is very different in different stages of the life cycle.

d. The form of the animal is different under different conditions—

- α. Daughter cells recently come from transverse division have a shape different from that of the products of longitudinal division or that of the ordinary individuals. It is not improbable that form varies according to the relation between growth and fission and the relative frequency of the two sorts of fission, longitudinal and transverse.
- β. Before and after and during sexual reproduction there are individuals whose form departs considerably from that of the ordinary individuals, being in general much more slender.
- γ. One finds occasionally swollen forms, as yet not understood, which may easily be mistaken for true species. *Cepedea "zelleri,"* for example, seems to be but a swollen form of *C. dimidiata.*
- δ. Ribbed, plicate, and twisted forms are often found. Sometimes all, or almost all, the individuals in an infection will show one of these conditions, suggesting specific diversity, but sufficiently wide comparisons seem to indicate that these are probably conditions due to physiological state.

e. Measurements of such characters as the intervals between the lines of cilia, or the number of cilia to the micromillimeter in the cilia lines, can be used only with caution, for at the anterior end of the body there are accessory interpolated lines of cilia decreasing the width of the interspaces, and the cilia in the lines may be more crowded anteriorly. After transverse division, the posterior daughter will show special transient conditions at its anterior end, and to a less degree the posterior end of the anterior daughter will show conditions of cilia arrangement different from that in ordinary individuals. Very well-nourished individuals may have the body somewhat distended and the lines of cilia will be further apart. Conversely, starved individuals may be shrunken with narrow intervals between the lines of cilia.

f. Individuals of different species may occur in the same individual host, and similarly we may have a double or multiple infection by two or more divergent strains of what might well be called one species.

It is well-nigh impossible in some instances to be sure in his comparisons that one has selected individuals which are in truly comparable physiological states and in similar stages in the life cycle. It would be a task of great magnitude and extreme difficulty to describe with any completeness and with discrimination the phenomena as they exist, and when such description had been given the determination of taxonomic divisions would doubtless still have to be artificial in numerous instances. All that will be attempted in the present paper will be to give an intelligible description of the chief data and to adopt the most plausible specific demarcations, but with the decided feeling that further study of wider data will necessitate modification of the present statement. A large part of the material studied was taken from preserved Anura and in some instances the parasites were poorly preserved. It is, however, surprising to see how usable such preserved material usually is. In general, it stains well, and often nuclear structure is well brought out, the chromosome number being readily determined. In numerous instances the material available is insufficient for as wide comparisons as would be desirable. In some cases only a single infection was found. All one can do is to describe the data found and recognize its limitations and the consequent tentative nature of some of the conclusions expressed.

In this study are included only the genera *Protoopalina*, *Zelleriella*, *Cepedea*, and *Opalina*. I believe the family Opalinidae should be restricted to these genera and not be stretched to include the very dissimilar and evidently not closely related astomatous genera such as *Discophrya*, *Hoplitophrya*, *Anoplophrya*, and others which have often been placed in this family. They are all forms with well differentiated macronuclei and micronuclei,[14] and clearly do not belong in the same family with *Protoopalina*, *Zelleriella*, *Cepedea*, and *Opalina*, in which there is no such divergent differentiation of nuclei. The differentiation of the nuclei in the Euciliata into macronucleus and micronucleus is almost, if not quite, the most remarkable morphological and physiological nuclear phenomenon in the animal kingdom. Indeed, we might well include the plants also in this statement. It seems, therefore, clearly unwarranted to classify with the Opalinidae the astomatous Euciliates merely on the ground that they, like the true Opalinids, have no ingestion apparatus. The latter quality is doubtless due, in all these genera, to their life as parasites and is a phenomenon of convergence rather than the result of genetic relationship.

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[14] The micronucleus of *Discophrya* has not been demonstrated, but the characteristic macronucleus of the Euciliata is present and probably a micronucleus is present. At any rate *Discophryo* is nearer to the Euciliata than to the Opalinidae.

In the year 1918[15] I twice published a classification scheme as follows:

- Ciliata
  - Protociliata
    - Opalinidae
      - *Protoopalina*
      - *Opalina*
  - Euciliata.

Further study of the material has shown that a truer expression of the actual conditions is given in the following classification, which is the one used in the present paper. The Opalinidae form an appendage to the Ciliata which may be grouped as follows:

- Ciliata
  - Protociliata
    - Opalinidae
      - *Protoopalininae*
        - *Protoopalina* (cylindrical, or slightly flattened, binucleate forms)
        - *Zelleriella* [new genus] (much flattened, binucleate forms)
      - *Opalininae*
        - *Cepedea* [new genus] (cylindrical or slightly flattened, multinucleate forms)
        - *Opalina* (much flattened, multinucleate forms)
          - *Opalinae angustae* (*occidentales*)
          - *Opalinae latae* (*orientales*).
  - Euciliata

The new genus *Zelleriella* is named for Ernst Zeller, who in 1877 published an excellent paper upon the species of Opalinidae then known. The new genus *Cepedea* is named for Casimir Cepede, whose studies of astomatous Ciliata have emphasized the fundamental distinction between true Opalinidae and other Ciliata.

The four genera of Opalinidae are reasonably distinct, not intergrading to the point of rendering the line of demarcation doubtful.

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[15] Metcalf, 1918 *a* and *b*.

The Protoöpalinas have in general two nuclei, though one known species is said to have but one, and one species has often as many as 6, 8, or even 10 nuclei. The nuclei are large, having in different species a major diameter of from 10 to 45½ micromillimeters. They have few chromosomes, from 4 to 10 massive chromosomes having been counted in different species. The granular chromosomes seem to show corresponding numbers. Nuclear conditions in *Zelleriella* resemble those in *Protoopalina*. In the mitosis of *Protoopalina* and *Zelleriella* the massive chromosomes are carefully halved and the daughter chromosomes of each pair are distributed to opposite daughter nuclei, whereas in *Cepedea* and *Opalina* some of the chromatin masses may remain undivided and pass bodily to one daughter nucleus, or it seems may even be extruded from the nucleus after being caught by the constricting nuclear membrane at the equator of the dividing nucleus. In *Cepedea* and *Opalina* the nuclei are more numerous, from four to several hundred. These nuclei are small, having a major diameter of from 2.6 to 7.75 micromillimeters, or in a very few species over 9 micromillimeters. The chromosomes are in general more numerous and this fact, together with the small size of the nuclei, makes it difficult to count them. *Opalina ranarum* and *Cepedea dimidiata* are thought by Neresheimer to have each twelve macrochromosomes.

Subfamily PROTOPALININAE.

The species of *Protoopalina* are circular, or nearly so, in cross section; the species of *Zelleriella* are flat, some being very thin, while others are intermediate in shape, being oval in cross section, with different proportional thickness in different species. There are corresponding shapes among the multinucleated forms, the cylindrical species belonging to the genus *Cepedea* and the much flattened species to the genus *Opalina*.

Perhaps the most remarkable feature of the *Protoopalininae* is that in many species the nuclei are regularly found to be resting in a midmitotic condition instead of in the reticulate condition characteristic of "resting" nuclei in general. The mitotic phase in which the nucleus comes to rest is different in different species of *Protoopalininae*. Only a few species have the nuclei characteristically in a reticulate condition. Most numerous are species whose nuclei are characteristically in an early or a late anaphase, or in an early, or a medium, or a late telophase. Equatorial plate nuclei are not characteristic of any species thus far studied, nor have nuclei in the skein stage been observed as the characteristic resting nuclei of any species, though this may be due only to the fact that in many species studied the preservation of the nuclei was not such as to allow clear analysis of the nuclear structure.

This departure in the *Protoopalininae*, and to a far less degree in the *Opalininae*, from the nuclear condition found in other animals and plants is most remarkable. In no other living things, so far as I know, do nuclei which have entered upon mitosis characteristically come to rest before they have completed the mitosis. This phenomenon was first observed in *Protoopalina* [" *Opalina* "] *mitotica* (Metcalf, 1912) and was later seen by Awerinzew (1913) in his *Protoopalina* [" *Opalina* "] *primordialis*. The present studies show its prevalence throughout this genus and also among the Zelleriellas. These mitotic phenomena give one important indication of the relationships between species. In a subsequent section of this paper the light these phenomena throw upon the question of the origin of the Ciliata and of the remarkable nuclear conditions in the Euciliata, will be discussed.

The several species of Opalinidae will first be described and I will then attempt a summary description of the genera, subgenera, and subfamilies.

A word of explanation as to the figures should be noted. Magnifications of 117, 460, 1,000, and 2,000 diameters are most used, and magnifications should be noted before comparing drawings. Many figures give merely the outlines of the body. Generally such figures show a number of drawings indicating the range of shape and size in the infection. Other figures add outlines of the nuclei, some including the macrochromatin masses. Cilia are in no case accurately drawn. They could not be except in highly magnified drawings, and then only in those cases in which fresh material has been available for study. In most instances the drawings do not indicate the fact that the cilia are more crowded over the anterior portion of the body. In the more detailed drawings the endospherules are in solid black while the ectospherules usually are not shown. In many of the figures the limits of the morphologically anterior end of the body are marked by two dots placed outside the contour of the body or its cilia.

Genus **PROTOOPALINA.**

Cylindrical or spindle-shaped *Protoopalininae*, circular, or nearly so, in cross section.

PROTOOPALINA PRIMORDIALIS (Awerinzew).

Opalina primordialis Awerinzew (1913).

Host.—*Rana nutti* Boulenger.

These *Protoopalinae* were found along with *Nyctotherus* in a single specimen of this frog fom Amani, German East Africa. Awerinzew's unillustrated description of this very interesting species is here given nearly in full. He says, in substance (comments by Metcalf enclosed in square brackets):

This species is of peculiar interest since in all individuals the nucleus is found to be in a stage of mitotic division. The infusorian has a slender elongated structure; its anterior end is rounded, its posterior end tapers somewhat. The body is covered with a mass of long cilia arranged in close set parallel furrows. The endoplasm is filled with an abundance of almost regular spheroidal inclusions [endosarc spherules of Metcalf]. There is always a single nucleus, which distinguishes this form sharply from *Opalina* [*Protoopalina*] *mitotica* recently described by Metcalf. In all my material I have found no individual whose nucleus is in the resting condition such as is known in related species. [This so-called resting condition in other species evidently means the reticulate condition, which is not the characteristic condition of any observed *Protoopalina* except *P. adelaidensis*. It is a transient phase of the mitosis, the nucleus resting in some other mitotic phase.] In my material the nucleus is always in two parts, lying in the longitudinal axis of the body and connected by a constricted portion of different lengths in different cases. In each half of the nucleus one can readily distinguish numerous strongly staining chromosomes of different lengths. The edges of the chromosomes are commonly somewhat irregular, as if toothed, but individuals are found with wholly smooth chromosomes. The chromosome number is evidently not always the same. [Doubtless the number of the definitive chromosomes is constant, but the number of chromatin masses may be different in different nuclei which are not in the middle anaphase condition, the stage of mitosis in which the definitive chromosomes may best be counted.] Most frequently I have found Opalinas with five chromosomes.

In cross section it is easily seen that the chromosomes are arranged along the outer edge of the nucleus, while in the interior of the nucleus, in the nodes of the network, lie very fine granules which stain noticeably more faintly than the chromosomes. The chromosomes, as the sections show, are not in actual contact with the nuclear membrane, but are separated from this by a layer of network ["*eine Wabenschicht*"]. In some cases the chromosomes are wholly distinct from one another, while in other instances they are connected into a ring by means of delicate weakly staining bridges as described by Metcalf for *Opalina* [*Protoopalina*] *intestinalis*.[16] [This indicates an early metaphase condition of these nuclei.]

The poles of both halves of the nucleus are pointed and appear wholly without granules, as does the narrow area connecting the two halves of the nucleus. Along the dividing nucleus are seen very numerous, weakly staining spindle threads.

At the beginning of the division of the infusorian, before the actual division of its nucleus, there follows a division and separation of the chromosomes, which renders clearly visible the spindle composed of achromatic[17] threads. Thereafter the nuclear bridge breaks, whereupon division of the body itself follows, so that each daughter cell once more enters upon a stage of protracted mitotic division. I have never observed longitudinal division of the Opalina here described. There is scarcely a necessity or possibility of such a division. [This statement is difficult to understand. In *Protoopalina intestinalis*, for example, nuclear mitosis is very similar to that here described by Awerinzew, and longitudinal as well as transverse fission of the body follows. I see no difficulty in its occurring in *P. primordialis.* If Awerinzew means merely that longitudinal fission does not occur because he failed to find it among the individuals in the single infection he observed, I would suggest that this is not unnatural. I have comparatively seldom seen longitudinal fission in freshly taken material, though I have studied material from many hundreds of infections of numerous species. I have however found many individuals with the beginnings of the longitudinal splitting showing as a slight indentation of the contour of the anterior end of the body, or appearing in a widening of the whole body. A slightly diagonal orienting of the two nuclei, one to the left, the other to the right, frequently accompanies the earliest stages of longitudinal fission and may be recognized by one familiar with the phenomena of fission in these animals.]

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[16] The best instance of this lateral union of the chromosomes during the early metaphase to form a ring has been described by Leger and Duboscq (1904, *b*, III) for *Protoopalina saturnalis* (see fig. 37, *a*, p. 64; compare fig. 4, *e*, p. 12).

[17] Metcalf (1909, also section 2 of this paper, p. 15) has described the chief fibers of the spindle as chromatic; that is, as filose pseudopodia from the chromosomes.

From the standpoint of the individuality hypothesis, which recognizes the several chromosomes as autonomous life-entities ["*Lebenseinheiten*"] the described case of a persistent existence of independent chromosomes, according to my conception, is most reasonable for the organism, for the organism thereby succeeds during the vegetative stage of the nucleus in avoiding the complicated progressive series of phenomena which involves a change in the nuclear structure and might well lead to a loss of independence of the chromosomes. [This sentence I have interpreted instead of translating. In German it reads:—"Vom Standpunkt der Individualitätshypothese, die die einzelnen Chromosomen als autonome Lebenseinheiten anerkennt, ist die beschreibene Fall einer beständigen Existenz von selbstständigen Chromosomen, meiner meinung nach, der für den Organismus am meisten rationale, da es ihm dadurch gelingt, während des vegetativen Stadiums des Kernes die komplizierten Progresse zu vermeiden, welche zu einer abänderung der Kernstruktur und zum Verlust der Selbstständigkeit der Chromosomen führt." In *Protoopalina intestinalis, P. caudata* and numerous other species whose nuclei, like those of *P. primordialis* rest in a midmitotic condition, the nuclei do go through a reticulate stage, which, however, is not prolonged. It would require detailed study of the nuclear cycle in *P. primordialis* to determine whether it does or does not have a similar transient reticulate stage. At any rate, the independence of the chromosomes probably results not so much from space relations, as Awerinzew implies, as it does from physico-chemical conditions.]

Since forms with nuclei constantly in the mitotic condition have been wholly unknown,[18] I consider the species of *Opalina* discovered by me to be new and I designate it *Opalina primordialis.* On the basis of a series of peculiarities which are possessed by different *Opalinae*, among which the nuclear structure is in part to be included, these animals must be separated to a wholly distinct group of cell-like organisms (=Protozoa), which possibly is related to the typical ciliate infusoria, but which can not be included merely as a separate family of the order Holotricha.

Awerinzew's description has been quoted nearly in full. As we go further with our description of the Protoopalinas we will see that their most salient character is the general prevalence among them of peculiar mitotic conditions such as have been described first for *P. mitotica* and later by Awerinzew for *P. primordialis.*

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[18] Conditions similar to those here described by Awerinzew were previously described by Metcalf (1912) for *Protoopalina mitotica,* as Awerinzew mentions above.

The general significance of this peculiar mitotic condition will be discussed later in this paper.

Awerinzew's description of his animals is not illustrated and measurements are not given. It is therefore not possible to make satisfactory taxonomic comparisons with other species. It is, however, evidently a distinct species, having its nucleus characteristically in an earlier mitotic condition than is the case in *Protoopalina diplocarya* which seems to be the species whose nuclei most resemble those of *P. primordialis.*

PROTOOPALINA DIPLOCARYA, new species.

Type.—United States National Museum Cat. No. 16428.

Host.—*Eleutherodactylus leptopus* (Bell). Cat. No. 15125 of the collections of the United States National Museum; from Mayne Harbor, Patagonia, collected February 5, 1877; length of individual host, 22 millimeters.

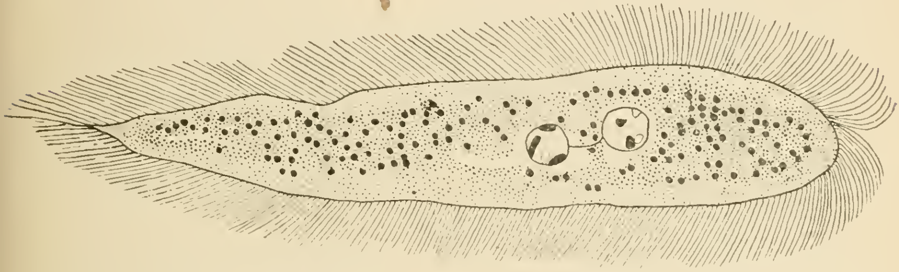

FIG. 11.—PROTOOPALINA DIPLOCARYA; AN OPTICAL, LONGITUDINAL SECTION. × 460 DIAMETERS.

But one specimen of the host was available. This had already been dissected. In its rectum was found an abundant infection of well-preserved Protoöpalinas.

Measurements of an ordinary individual.—Length of body 0.217 mm.; greatest width of body 0.0413 mm.; thickness of body at same level 0.038 mm.; diameter of nucleus 0.013 mm.; diameter of endoplasmic spherules 0.002 mm.; width of average interval between the lines of cilia, at the anterior end of the body 0.00125 mm., near the posterior end of the body 0.0035 mm.

The lance form of the body and the very long cilia are shown in figure 11. The whole body is clothed with cilia except the acuminate, often spinelike, posterior tip, which is naked. The cilia are three times more numerous over the anterior quarter of the body, a fact not shown in the figure. The ectosarc is quite thick and is more coarsely alveolated than the endosarc, a fact not shown in the figure. There are numerous spheroidal spherules in the endosarc, in which also may be seen a well developed excretory vacuole, especially well developed in the neighborhood of the nuclei. The two nuclei are spherical, or very nearly so, and are near together. They are always connected by a thread. In more than a thousand individuals observed no case of mitosis was found, nor any instance of fission. In many individuals I was able to count four chromatin masses in each nucleus. In a few other individuals each nucleus showed eight chromatin masses. Six and seven chromatin masses also were found, also instances of a larger number of smaller chromatin bodies. These conditions indicate four as the probable number of the massive chromosomes, but it is unsafe to say positively without observation of the anaphase stage of mitosis, the stage when the chromosomes are most characteristic in form and most distinctly seen. I have no nuclei in this condition to observe.

This species is similar to Raff's *Protoopalina acuta*, soon to be described, but differs in its broader form; in its broadly rounded anterior end; in its abruptly acuminate, almost spinelike, posterior extremity; in the greater length of its cilia; in the presence of cilia, apparently of full length,[19] over all of the body, posteriorly as well as anteriorly; in the approximation of its nuclei; in their larger size and spherical, rather than ellipsoidal, form; in the thread which always connects them.

Protoopalina diplocarya still more resembles *P. dorsalis* (Raff) in form, but its cilia are much longer than those of the latter species; it is more nearly circular in cross section; its nuclei are nearer together than in *P. dorsalis* and they are always united by a thread.

P. diplocarya differs from *P. papuensis*, next to be described, in being less elongated posteriorly and especially in the condition of its nuclei, which are spherical instead of pear-shaped. The internal condition also is different as the description of *P. papuensis* will show.

PROTOOPALINA PAPUENSIS, new species.

Type.—United States National Museum Cat. No. 16625.

Host.—*Hyla dolichopsis* (Cope), one abundant infection of well-preserved parasites, in United States National Museum specimen No. 57718, from Sorong, Dutch Papua, 1906; Julius Hurter, sr., collector.

Measurements of an ordinary individual.—Length of body 0.315 mm.; width of body 0.048 mm.; length of nucleus 0.023 mm.; width of nucleus 0.012 mm.; diameter of endospherule 0.0015 mm.; cilia line interval, anterior 0.002 mm., posterior 0.004 mm. In an individual in an early stage of fission the dumb bell-shaped nuclei are 0.053 mm. long and 0.009 mm. wide at the widest part. The cilia are not well enough preserved to measure. Macrochromosomes 4.

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[19] Usually in Opalinids collected from frogs which have been long preserved one finds the cilia in quite imperfect condition. But even in many such cases, when the cilia are matted together, the width of the peripheral band composed of matted cilia, and the direction of the cilia in this band, indicate roughly the length of the cilia. In the specimens of this species studied the cilia are fairly clear.

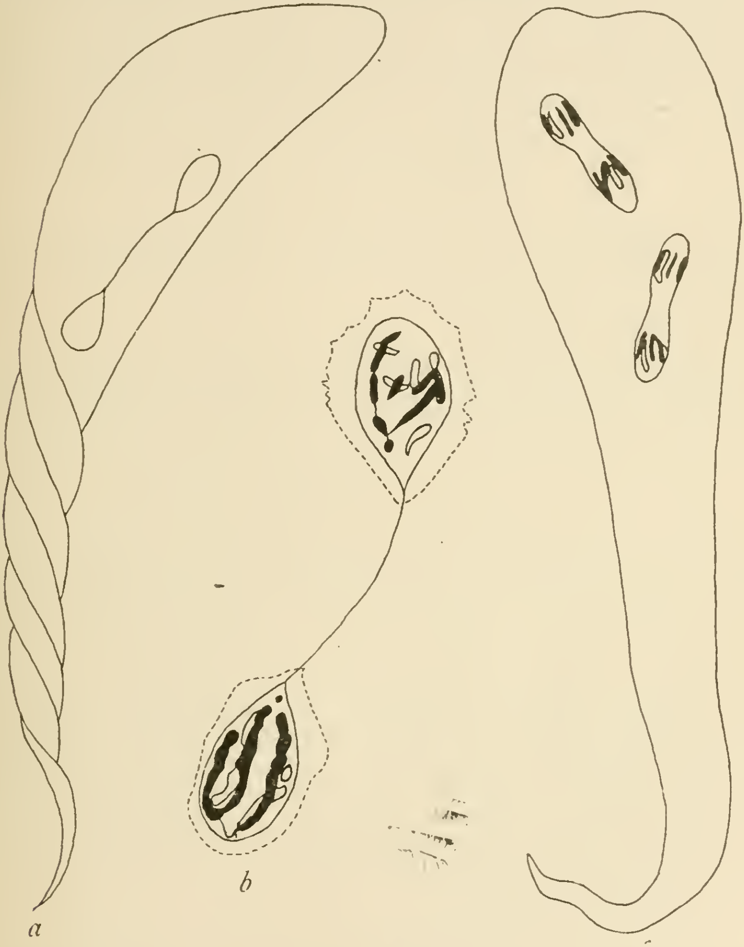

Fig. 12.—Protoopalina papuensis; *a*, an individual of the ordinary sort, × 460 diameters; *b*, its nuclei, × 1,000 diameters; *c*, an individual entering upon fission, × 460 diameters.

This *Protoopalina* is similar in shape to a macrogamete mother cell of *P. intestinalis*, resembling in this regard *P. diplocarya* or *P. acuta*, but its nuclei are very different from those of the latter two species. Like *P. acuta*, it tapers posteriorly to a very elongated, slender point, which is devoid of cilia, and is spirally grooved, appearing as if spirally twisted. The pear-shaped nuclei are quite far apart but are connected by a slender thread. In the great majority of individuals each nucleus is in a skein stage of mitosis, closely similar to the same stage in *P. intestinalis.* The nuclei are thus seen to be more advanced in mitosis in their internal structure than are those of *P. diplocarya*, for though still pear-shaped they have their macrochromosomes usually united into a skein. In individuals which are entering upon fission there is no longer a thread connecting the two nuclei. Each of these is much elongated and dumb-bell shaped, and in each end of each dumb-bell are clearly seen four macrochromosomes.

The species is very distinct from any other known form.

PROTOOPALINA ACUTA (Raff).

Opalina acuta Raff (1912).

Host.—*Limnodynastes dorsalis* (Gray), from Australia: One infection observed by Raff.

Raff's description in full is:

> This other new species from *Limnodynastes dorsalis* is much smaller than *O. dorsalis*, and tapers very much toward the posterior end, forming a long tail-like process. The average length is 257μ and the breadth at its widest portion of about 38μ. The anterior nucleus is situated about 57μ from the front end and the posterior about 10μ further on, and generally slightly nearer the ventral [!] surface. The cilia are well developed on the body portion, but gradually diminish in size and number on the posterior process until for a length varying from 25μ to 50μ from the tip of the process there appear to be none. As the animal moves along, the anterior end is slightly raised, and the dorsal surface appears flattened or straight, while the ventral surface is curved. The characteristic feature that distinguishes this species from others is the very long, thin posterior portion.

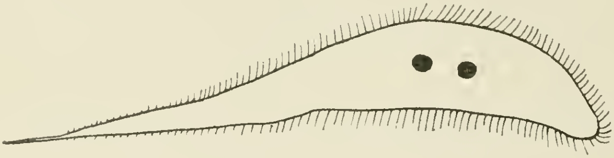

Fig. 13.—Protoopalina actua. (After Raff.)

The description of *Protoopaleria xenopodos* (p. 61) should naturally follow here, but is postponed because of certain comparisons of nuclear conditions.

PROTOOPALINA DORSALIS (Raff).

Opalina dorsalis Raff (1912).

Host.—*Limnodynastes dorsalis* (*Gray*), from Australia. One infection observed by Raff.

Raff's description in full is as follows:

> The species of *Opalina* previously found in *Limnodynastes dorsalis* are *O.* [*P.*] *intestinalis* and *O.* [*Zelleriella*] *binucleata*, the latter being a new

one that I described in part 1. In a frog of the same species just examined, however, I found neither of these present, but two different ones, both of which seem to be new. They are binucleated forms, and were present in great numbers along with *Nyctotherus cordiformis.* The larger of the two I propose to call *O. dorsalis.* It is elongate in form and has a very broad anterior end, is slightly flattened and swims along on either side, giving an occasional roll over onto the other side as it progresses. This enables us to see a thin edge which indicates that the animal is not circular in cross section, but broadly oval. It is ciliated all over with very long cilia, and the two nuclei are always situated in the anterior half of the animal. The most distinguishing feature of the species is the greater width of the anterior end as compared with the posterior. It tapers gradually toward the hinder end and terminates in a point. The cilia extend right to the posterior end. The average length is between 358μ and 430μ and at its widest portion it measures about 72μ. The one drawn in the figure shows the characteristic cork-screwlike folds that the animal presents when moving along. It also shows the slight turn of the "head" end that is noticed during locomotion.

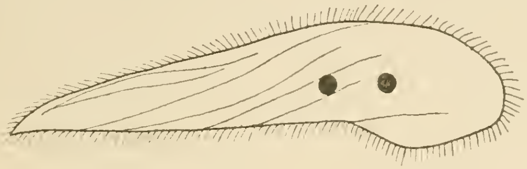

FIG. 14.—PROTOOPALINA DORSALIS. (After Raff.)

The species at first sight resembles *O. intestinalis* slightly in outline and position of the nuclei, but it differs from it in that the anterior end is broader in proportion to the posterior, which tapers more and ends more sharply than in *O. intestinalis*, and in that the body is slightly flattened and so would not be circular in cross section.

PROTOOPALINA PERONII, new species.

Type.—United States National Museum Cat. No. 16620.

Host.—*Limnodynastes peronii* (Dumerîl and Bibron), one scant infection from United States National Museum specimen No. 62748, from Wandandian, New South Wales, Australia, collected by C. M. Hoy, August 1, 1919. Cleland and Johnston (1910) report finding an unidentified species of "*Opalina*" [it might have been either a *Protoopalina* or *Zelleriella*, not an *Opalina* in our present terminology] in this species of host.

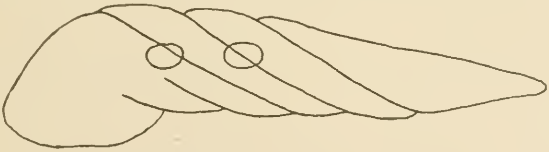

FIG. 15.—PROTOOPALINA PERONII, × 460 DIAMETERS.

Measurements of an ordinary individual (*fig. 15*).—Length of body 0.16 mm.; width of body at the wide anterior end 0.0326 mm.;

thickness of body at the level of the nuclei 0.02 mm.; length of nucleus 0.0108 mm.; width of nucleus 0.0065 mm.; diameter of endospherule 0.002 mm.

My specimens of this species are less than half as long as those of Raff's *Protoopalina dorsalis* and they have ellipsoidal instead of spherical nuclei. They resemble *P. dorsalis* closely in the form of the body, being broad in front, being considerably flattened and having the body spirally twisted. Raff's *Protoopalina acuta* has oval nuclei, but its body tapers posteriorly to a slender, elongated point. *Protoopalina peronii* resembles *P. dorsalis* in body form, while its ellipsoidal nuclei resemble those of *P. acuta.* It seems intermediate between these two species. There are a few cysts present in the infection so it is probable that material gathered at a different time of year would show individuals larger than those in our material.

PROTOOPALINA PELOBATIDIS, new species.

Type.—United States National Museum Cat. No. 16429.

Host.—*Pelobates fuscus* (Laurenti), United States National Museum specimen No. 37191, 54 mm. long, from Klosterneuburg, Austria, May 1897. F. Werner collector; also No. 16450, 48 mm. long from "Europe," no data. Two other individuals from this locality were opened, but no Opalinids were found. Also two individuals collected at Turin, Italy, were opened without finding Opalinids. These bore no data, but the card bore the words "Roy. Zool. Mus." They were soft and in very poor condition, and this may account for the absence of Opalinids.

Measurements.—Length of body 0.097 mm.; width of body 0.0185 mm.; length of each ellipsoidal nucleus 0.0105 mm.; width of each ellipsoidal nucleus 0.0068 mm.; diameter of endospherules 0.00175 mm.; cilia line interval, anterior end 0.0023 mm., middle of body 0.0035 mm., posterior end 0.00375 mm. The number of massive chromosomes appears to be 8 or perhaps 10.

This is a slender species rather similar to *Protoopalina intestinalis*, but is much smaller and differs in nuclear condition. *P. intestinalis* has been reported from *Pelobates fuscus.* Göze's reference (1782) is not sufficient for specific identification, nor is Kent's (1881–82), nor Balbiani's (1887), nor Bütschli's (1887–1889). Indeed, I do not find any reference to *P. intestinalis* from *Pelobates* which is sufficient to enable us to determine whether the *intestinalis*-like form reported was true *intestinalis*, or was the species here described.

The body is rounded in front. The posterior portion of the body narrows, not gradually but rather abruptly to a rounded point. There is no trace of any posterior "spine." The two nuclei are generally pear-shaped and nearly always connected by a thread. They are apparently always so connected except in individuals just about to divide by fission. In the ordinary individuals each nucleus shows a group of chromatin masses of varying number, lying peripherally

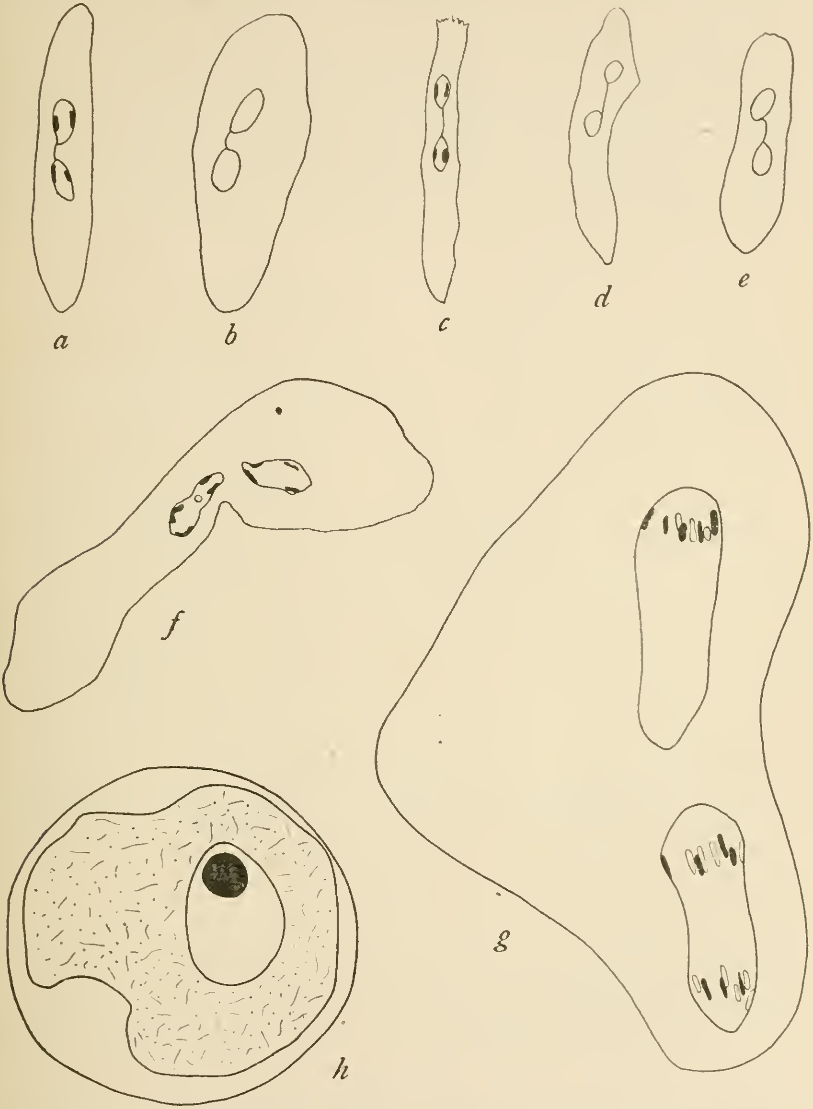

Fig. 16.—Protoopalina pelobatidis; *a* to *e*, individuals in the usual condition; *f*, an individual in transverse fission, with anaphase nuclei; *g*, longitudinal fission. (Note 8 macrochromosomes. These are omitted in one end of one nucleus which was overlaid by débris); *h*, a cyst; *a* to *f*, magnified 460 diameters; *g* and *h*, magnified 2,000 diameters; *a* to *f* have the anterior end toward the top of the page; in *g* the already double anterior end is toward the right.

about in the equator of the nucleus (fig. 16, *a* and *c*). The nuclei in this condition may be rather wide, but are more often more slender. In general they are noticeably more slender than in *P. intestinalis*.

About 8 per cent of the individuals in the infections studied have each of the two nuclei in active division, preparatory to division of the body. Some of these nuclei are in an anaphase of mitosis, and these show apparently 8 or possibly 10 massive chromosomes (fig. 16, *g*). The material is neither sufficient nor sufficiently well preserved to permit entire confidence in the counts. The two anaphase nuclei are slenderly elliptical, or even slightly dumbbell-shaped, and may or may not still be connected by a thread. The proportion of all individuals in *P. pelobatidis* which have their two nuclei connected is much larger than in *P. intestinalis.*

PROTOOPALINA CAUDATA (Zeller).

Opalina caudata Zeller (1877).

Hosts.—Bombina bombina (Linnaeus) [=*Bombinator igneus* Laurenti] and *Bombina pachypa* (Bonaparte) [=*Bombinator pachypus* Bonaparte], from central and southeastern Europe. It has been reported from a single specimen of *Bufo viridis* Linnaeus from Naples, Italy, and I shall describe four infections from *Discoglossus pictus*, Otth, which I place as a variety of this species.

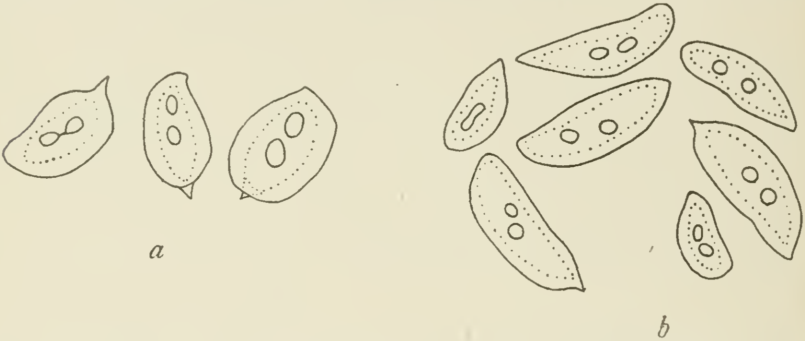

Fig. 17.—Protoopalina caudata, form lata, from Bombina pachypa; the two groups are from different hosts: the sharp-pointed ends are posterior: *a*, extreme lata forms; *b*, less extreme forms which should probably be classed as lata. × 117 diameters. The dotted lines indicate the boundary between ectosarc and endosarc.

This species of *Protoopalina* occurs in two quite markedly dissimilar forms, one slender, the other stocky. These would be regarded as distinct species, were it not for the complete intergradation between them, and also for the fact that similar slender and swollen forms are known in other species. as for instance, *P. saturnalis* and *Cepedea dimidiata.* The forms are so divergent that they deserve distinct names and separate description.

Specimens of *P. caudata* have been deposited with the United States National Museum. See Cat. Nos. 16432 (from *Bombina bombina*) and 16433 (from *Bombina pachypa*).

Form LATA, new.

Type.—United States National Museum Cat. No. 16430.

Measurements.—Length of body 0.2 mm.; width of body 0.095 mm.; length of nucleus 0.04 mm.; width of nucleus 0.026 mm.; length of posterior process 0.062 mm.; cilia line interval, first individual, anterior 0.0019 mm., posterior 0.0031 mm., second individual, anterior 0.0019 mm., posterior 0.0031 mm., third individual, anterior, 0.0018 mm., posterior 0.00357 mm.; thickness of ectosarc 0.0161 mm.

The nuclei in these swollen forms are often in active division.

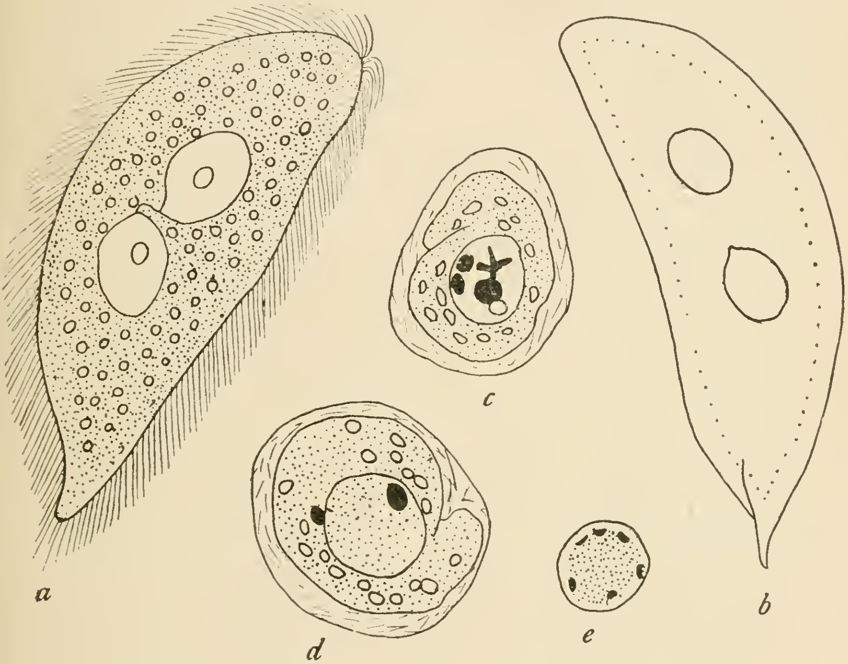

Fig. 18.—Protoopalina caudata; from Bombina pachypa: *a* and *b* are specimens which should probably be classed as form lata; *c* and *d* are cysts; *e*, is an optical transverse section of a nucleus in an anaphase of mitosis, showing six macrochromosomes; *b* is magnified 460 diameters, the other figures 1,000 diameters.

Form ATTENUATA, new.

Type.—United States National Museum Cat. No. 16431.

Measurements.—Length of body 0.1543 mm.; width of body 0.024 mm.; length of nucleus 0.0152 mm.; width of nucleus 0.008 mm.; length of endospherule 0.002.; width of endospherule 0.0018 mm.; cilia line interval, anterior 0.0025 mm., middle 0.0029 mm., posterior 0.003 mm.

These slender individuals are much smaller than *Protoopalina caudatá lata.* In form they somewhat resemble *P. intestinalis,* but they taper posteriorly, sometimes very gradually, ending in a spine-like tip. Their nuclei are much smaller than those of *P. caudata lata* and are resting in a much earlier phase of mitosis. The two pear-shaped nuclei are united by a thread and each is seen to be in an anaphase condition.

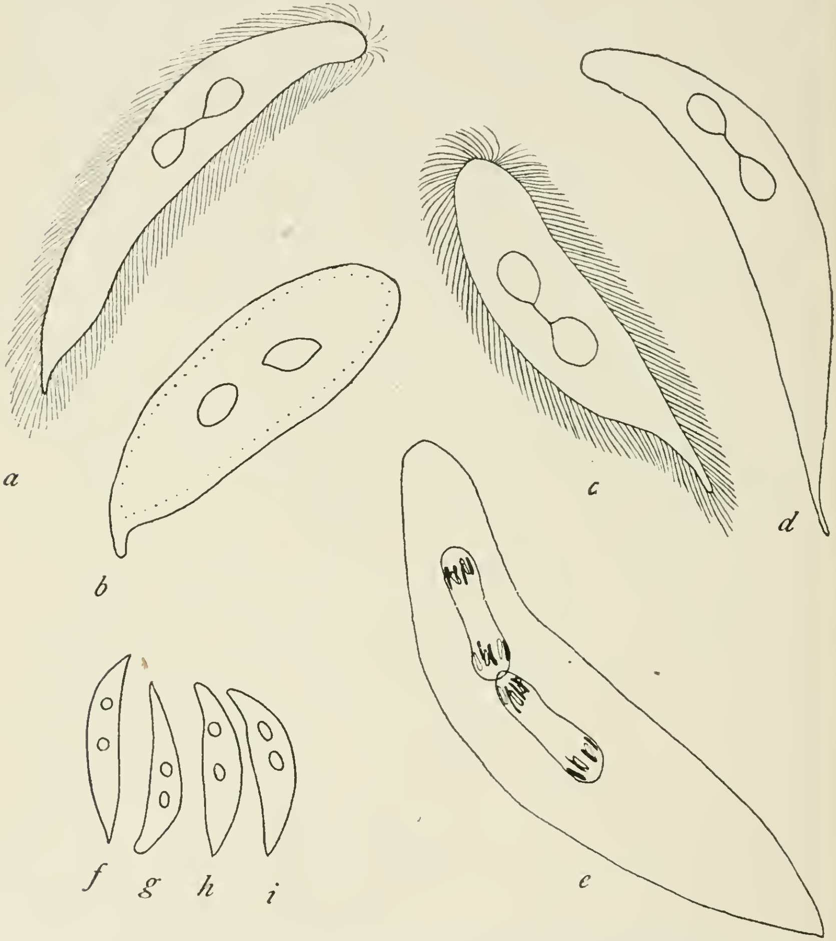

FIG. 19.—PROTOOPALINA CAUDATA, MOSTLY FORM ATTENUATA, FROM BOMBINA BOMBINA; *b*, *e*, AND *i* ARE ENTERING ON DIVISION; *a* TO *e*, MAGNIFIED 460 DIAMETERS; *f* TO *i*, MAGNIFIED 117 DIAMETERS. THE ANTERIOR ENDS ARE ABOVE EXCEPT IN *g*.

INTERMEDIATE FORMS.

Strange as it seems, at first glance, to include the two forms just described in the one species *caudata*, they so completely intergrade that there seems no alternative. Reference to the accompanying figures makes this evident. In figure 19, *a* and *d*, are seen individuals from *Bombina bombina* which are almost typical *attenuata*. In figure 20 are less slender forms. In figure 18 the animals are considerably more stocky and do not taper gradually to the posterior tip. Figure 17, *b*, shows individuals almost as stocky as extreme *caudata lata* such as are drawn in figure 17, *a*.

Some of these intermediate forms of *P. caudata* closely resemble *P. intestinalis*, but the infections can be distinguished by the smaller size of the individuals; by the fact that many bear posterior "spines" and others are at least sharply pointed posteriorly; and especially by the fact that in these, as in all forms of *P. caudata*, the number of massive chromosomes is six (figs. 18, *e* and 19, *e*), while in *P. intestinalis* there are eight (figs. 3 and 4, *d*). *Protoopalina caudata*, with its diverse forms, well illustrates the danger of specific description of Opalinids from limited material. Yet in this paper numerous species must be described and named on the basis of one or only a few infections.

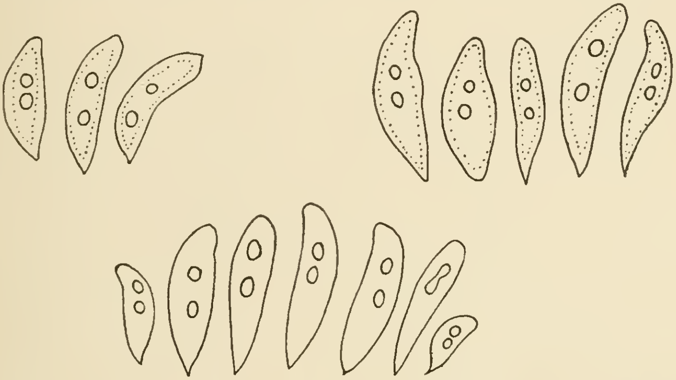

FIG. 20.—PROTOOPALINA CAUDATA, FROM BOMBINA PACHYPA; EACH GROUP WAS TAKEN FROM A SEPARATE HOST. × 117 DIAMETERS. THESE ANIMALS INTERGRADE BETWEEN THE FORMS ATTENUATA AND LATA. THE ANTERIOR ENDS ARE ABOVE IN ALL FIGURES EXCEPT THE THIRD IN THE UPPER LEFT-HAND GROUP.

I do not see that we have data to indicate the interpretation of the broad and narrow forms in this species. There seems nothing to show that the narrow individuals give rise to males and the broad individuals to females, nor are there observations to associate the narrow animals with a preponderance of longitudinal division and the broader animals with an especial prevalence of transverse fission.

Specimens of these intermediate forms have been deposited with the United States National Museum as Cat. No. 16434.

PROTOOPALINA CAUDATA DISCOGLOSSI, new subspecies.

Type.—United States National Museum Cat. No. 16438.

Host.—*Discoglossus pictus* Otth; two infections, *a*, in United States National Museum specimen No. 10052 from Sardinia, the type infection, and *b*, United States National Museum specimen No. 37193, from Eastern Algeria (for its Opalinid see United States National Museum Cat. No. 16437).

Measurements.—Length of body 0.1469 mm.; width of body 0.0254 mm.; length of nucleus 0.013 mm.; width of nucleus 0.0087 mm.; diameter of endospherule 0.0024 mm.; cilia line interval, anterior 0.00125 mm.; middle 0.0035 mm.; posterior 0.0035 mm.

These animals are shaped in general like *P. intestinalis*, but posteriorly they end in a distinct point, or more often in a short "spine" which is often curved as it is in *P. macrocaudata*, still to be described.

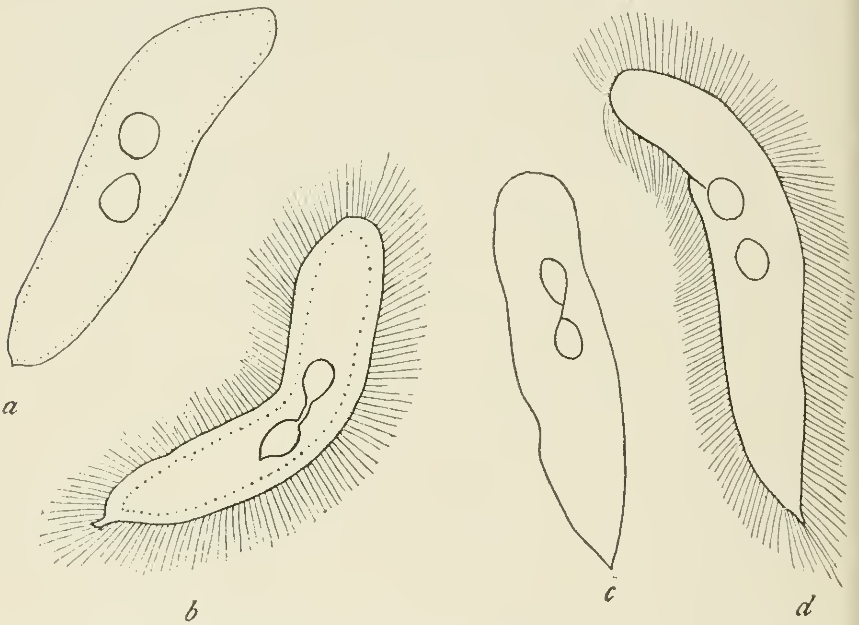

FIG. 21.—PROTOOPALINA CAUDATA DISCOGLOSSI. × 460 DIAMETERS.

They resemble also some of the intermediate forms of *P. caudata*, but have more numerous cilia over the anterior end of the body (not shown in the figures), and their nuclear conditions are more diverse. Two distinct nuclei are most usual, and these lie quite far back in the body. In individuals recently come from division, the nuclei are united by a thread, or we may even find cases of dumb-bell-shaped nuclei, with the constriction very marked. None of my material is well enough preserved to allow counting the chromosomes.

Protoopalina caudata has not been reported from *Discoglossus*, but *P. intestinalis* has. It is possible that the form here described is the one that has been reported as *P. intestinalis*.

PROTOOPALINA NUTTI, new species.

Type.—United States National Museum Cat. No. 16621.

Host.—*Rana nutti* Boulenger, United States National Museum No. 41438, from British East Africa, collected October, 1906, by the Smithsonian African Expedition on "the Mount Kenia trip." Of nine frogs collected at the same time and place eight were infected with this *Protoopalina.*

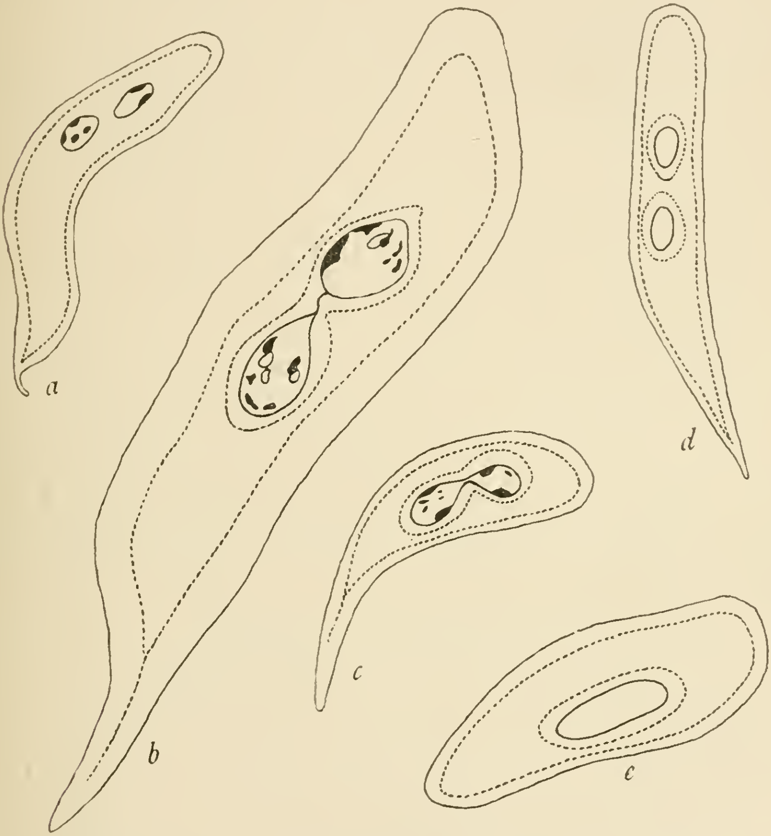

FIG. 22.—PROTOOPALINA NUTTI: *a, b, c,* AND *d,* SLENDER INDIVIDUALS; *e,* A BROADER FORM; *b,* MAGNIFIED 1,000 DIAMETERS, THE OTHER FIGURES 460 DIAMETERS.

Measurements of an ordinary individual.—Length of body 0.126 mm.; width of body 0.0258 mm.; length of nucleus 0.013 mm.; width of nucleus 0.0085 mm.; length of cilia at least 0.015 mm. (The cilia are seldom well enough preserved for one to be sure he is measuring their full length.)

This species is elongated. Usually the posterior end of the body tapers evenly to a long point (fig. 22, *c* and *d*), giving much the shape of a macrogamete mother cell. Other individuals narrow more abruptly, giving the posterior end of the body the appearance of a spine-like process such as is found in numerous species (fig. 22, *a*). The posterior point is rounded, not sharp, at the tip. The posterior end of the body is devoid of cilia. The cilia are long. The ectosarc is rather thick.

The nuclear condition is different in different individuals, the conditions divergent from the mean being shown in a larger proportion of the individuals than in most species. Usually there are two independent, ellipsoid nuclei rather near together, the posterior one usually near the middle of the body (fig. 22, *d*). In some individuals the nuclei are further forward (fig. 22, *a*), these individuals probably having come from posterior daughter cells at the last transverse division. Numerous individuals are found with dumb-bell-shaped nuclei, having evidently recently come from fission (fig. 22, *c*). We find all stages between these dumb-bell nuclei and the usual pair of ellipsoidal nuclei. Division of the body occurs when each of the two nuclei are much elongated or are dumb-bell-shaped (fig. 22, *e*, a daughter cell). One evidently abnormal individual was found which had three greatly constricted, slender dumb-bell nuclei.

The histological condition of the nuclei is not such as to allow detailed study. The number of macrochromosomes seems to be four or eight. Many nuclei show four chromatin masses, others show eight. One can not be quite sure from the preparations that the larger number of chromatin masses, in the latter nuclei, is due to fragmentation of four chromosomes during the metaphase of mitosis, as is often seen in other species and as is probable in this species.

Protoopalina nutti resembles in shape *P. caudata* form *attenuata*, but in the former species the nuclei are not placed so far forward in the body, the nuclear conditions are more various, or rather the proportion of individuals with nuclei divergent from the mean is greater, and the average nuclear condition is not the same, the nuclei being ellipsoidal and not pointed at one end, also the cilia are longer, while the macrochromosome number appears not to be six. This species is not identical with *P. orientalis*, which has eight macrochromosomes, for in the latter species the body is less tapering posteriorly. Awerinzew's *P. primordialis*, from the same species of host and the same general geographic region, is not sufficiently described for identification, but it can not be *P. nutti*, for Awerinzew said each individual in his material had but one nucleus, this being dumb-bell-shaped.

In the same individual host (United States National Museum No. 41438), along with the slender Protoöpalinas described, are a number of huge individuals a little longer than the slender ones and three to four times as thick (figs. 22, *A*, and 23). They must have 10 to 15 times the bulk of the slender forms. These swollen individuals probably belong to the species *P. nutti* and are probably comparable to the greatly enlarged individuals of *P. caudata* sometimes found. These large individuals of *P. nutti* resemble the slender individuals in having a thick ectosarc and in having the perinuclear vacuoles well developed. The cilia are apparently of the same length. Though the body is so stocky, some individuals show slight but definite indication of a posterior point which, as in the slender forms, is not sharp but rounded at the tip.

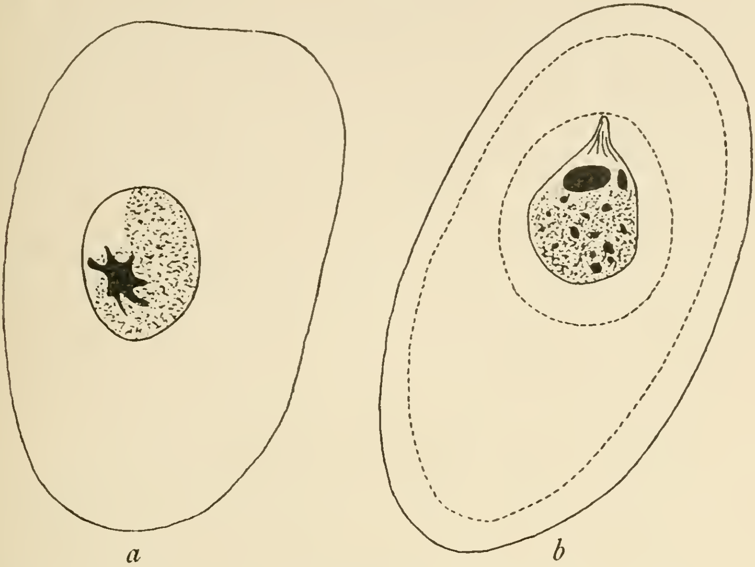

FIG. 22*A*.—PROTOOPALINA NUTTI, LARGE INDIVIDUALS, APPARENTLY ABNORMAL; × 460 DIAMETERS.

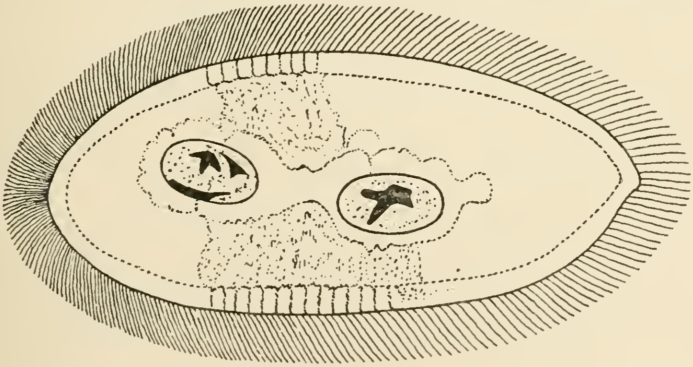

FIG. 23.—PROTOOPALINA NUTTI, STOCKY FORM. THE NUCLEI OF THIS AND OTHER STOCKY INDIVIDUALS SEEM ABNORMAL. × 460 DIAMETERS. THE ANTERIOR END IS TO THE LEFT.

The histological condition of the nuclei suggests that these large individuals are not normal. One was found with three medium-sized nuclei, one of which had extruded two chromatin masses into the perinuclear vacuole. Several others were found with but a single nucleus. In all the large individuals the massive chromatin of the nuclei was aggregated into apparently abnormally large clumps and the whole appearance of the nucleus was abnormal. The individual with three nuclei was a bit more slender than is usual for these swollen individuals. Its nuclei, too, were intermediate in size and condition between the nuclei of the slender individuals and those of the large individuals.

Measurements of an ordinary binucleated individual of the larger sort.—Length of body 0.174 mm.; width of body 0.076 mm.; length of nucleus 0.03 mm.; width of nucleus 0.0174 mm.

PROTOOPALINA ORIENTALIS, new species (fig. 24, p. 49).

Type.—United States National Museum Cat. No. 16460.

Host.—*Bombina* [*Bombinator*] *orientalis* (Boulenger), 10 infections. The type infection is from United States National Museum specimen No. 17527, 42 mm. long, from Fusan, Korea, 1885, P. L. Jouy, collector. There are two other infections from the same set of specimens, and there are seven infections from the Yulu River, Southern Manchuria, China, A. de C. Sowerby, collector.

Measurements of a large individual.—Length of body 0.288 mm.; width of body 0.063 mm.; length of nucleus 0.0236 mm.; width of nucleus 0.0133 mm.; diameters of endospherules, respectively, 0.0016 mm., 0.0022 mm., 0.0025 mm.; cilia line interval, anterior 0.0019 mm., posterior 0.004 mm.

Measurements of a small individual.—Length of body 0.187 mm.; width of body 0.043 mm.; length of nucleus 0.02 mm.; width of nucleus 0.01 mm.

As in *Protoopalina caudata*, so also in *P. orientalis* we find stocky (fig. 24, *c*), and more slender (fig. 24, *b*) forms and intergrades between the two, though the extremes in either direction are not so much emphasized as they are in *P. caudata.* In most individuals the body is sharply pointed posteriorly. In some cases the point is not seen, the posterior end being rounded (fig. 24, *k* and *l*), as it is sometimes in *P. caudata.* The two nuclei are connected by a thread, as shown in the figures, except in individuals ready for fission, and except in daughter cells recently come from fission, in which we see a dumb-bell-shaped nucleus (fig. 24, *c*). The cytological condition of the nucleus has not been studied.

PROTOOPALINA MACROCAUDATA, new species.

Type.—United States National Museum Cat. No. 16439.

Host.—*Bombina* [*Bombinator*] *orientalis* (Boulenger), United States National Museum specimen No. 17529, female with eggs, 42 mm. long, from Fusan, Korea, 1885, P. L. Jouy, collector.

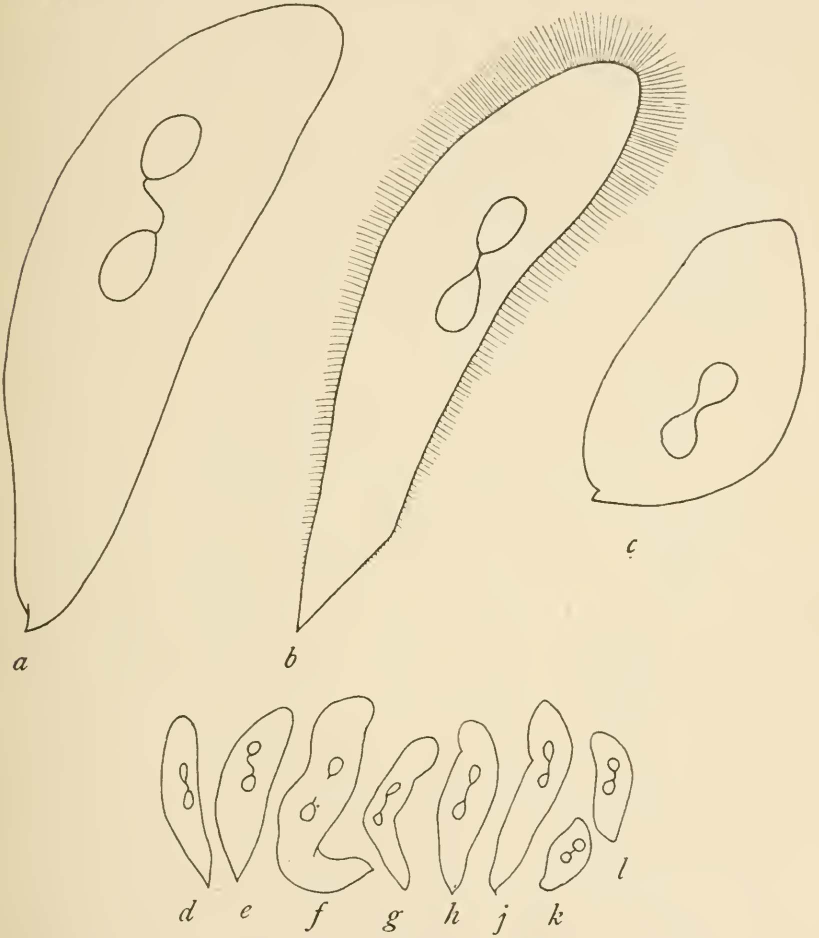

FIG. 24.—PROTOOPALINA ORIENTALIS; *a* AND *b*, USUAL CONDITION; *c*, A DAUGHTER CELL; *d* TO *l*, VARIETY IN FORM AND SIZE. *a* TO *c*, MAGNIFIED 460 DIAMETERS; *d* TO *l*, MAGNIFIED 117 DIAMETERS. THE ANTERIOR ENDS ARE UP IN ALL FIGURES.

Measurements—Length of body 0.09 mm.; width of body 0.074 mm.; length of posterior process 0.0217 mm.; length of nucleus 0.03 mm.; width of nucleus 0.0142 mm.; diameter of endospherule 0.0025 mm.; cilia line interval, anterior 0.00266 mm., posterior 0.0036 mm.

This Opalinid, though much smaller, resembles *P. caudata* form *lata* in its appearance, but its nuclei bear eight instead of six macrochromosomes. Its posterior, spine-like process is large and is curved. The species seems sharply distinct, but it would be desirable to compare very fully numerous infections of this species and of *P. orientalis*, to be entirely sure that it does not intergrade with the latter species as the broad *caudata* grades into the attenuate form.

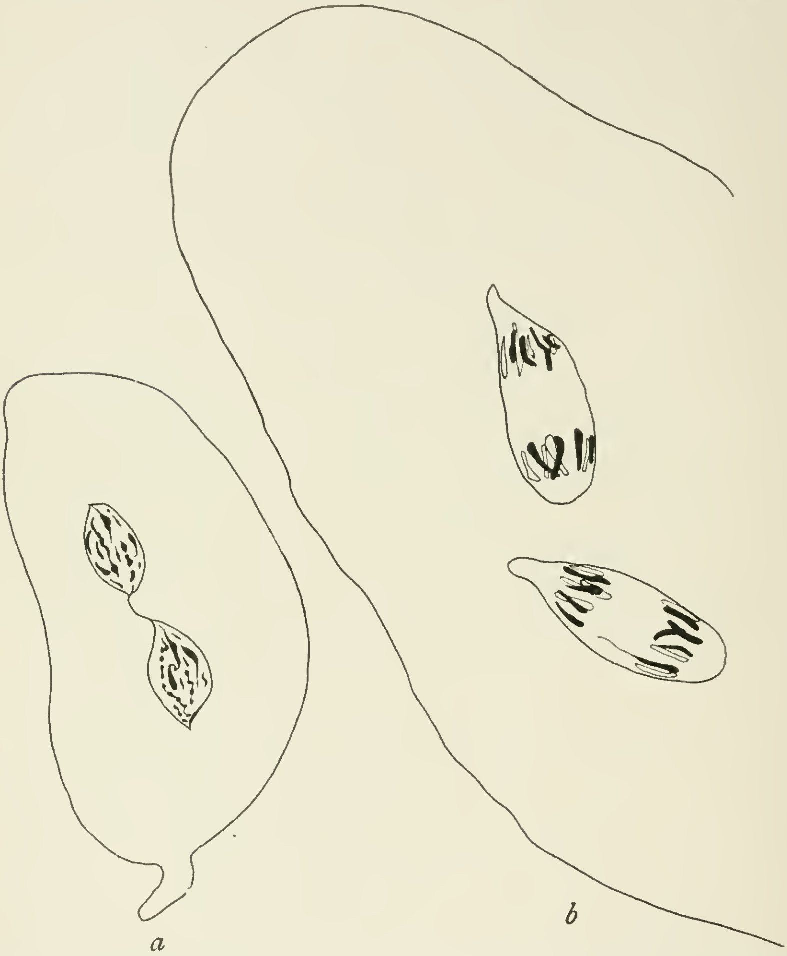

FIG. 25.—PROTOOPALINA MACROCAUDATA; *a*, IN THE ORDINARY CONDITION. × 460 DIAMETERS; *b*, IN AN ANAPHASE OF MITOSIS, × 1,000 DIAMETERS.

PROTOOPALINA STEVENSONI, new name.

This species was described, but not named, by Stevenson (1911).

Host.—Bufo regularis, from Khartoum, Sudan.

The measurements taken from Stevenson's figure of the largest individual are as follows.—Length of body 0.08 mm.; width of body 0.06 mm.; length of nucleus 0.013 mm.; width of nucleus 0.0077 mm.

Stevenson's description in full is as follows:

> *Nytotherus cordiformis* and a *Balantidium* are both common, but instead of *Opalina* we have another organism something of the same class though it differs in its nuclear arrangement. The largest specimens of this are about 80μ by 60μ, while the smallest are about 60μ by 16μ. They are covered by cilia of a uniform length, have no apparatus for ingestion or egestion, and the nucleus usually consists of two pear-shaped bodies connected by a definite narrow strand. Up to the present I have not identified this. It is characterized by having a definite spine at one end, especially in the smallest and middle-sized forms.

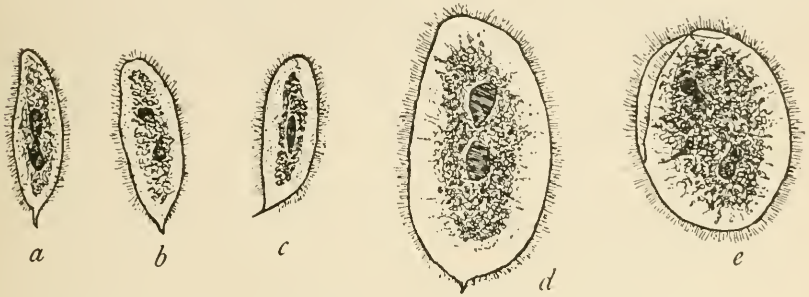

Fig. 26.—Protoopalina stevensoni, × 450 diameters. (After Stevenson.)

All of Stevenson's drawings of this form are given in the accompanying figure 26. Close relationship to *P. caudata* is very evident. In my abundant material from *Bufo regularis* I was not fortunate enough to find these animals.

PROTOOPALINA INTESTINALIS (Stein).

??*Hirudo intestinalis* Bloch (1782),
"*Flimmerwalzen*," *Leucophra*, Göze (1782),
Bursaria intestinalis Ehrenberg (1831),
Opalina intestinalis Stein (1856).

The general description of a *Protoopalina*, in Section 2 of this paper, is based upon *P. intestinalis*. To this must be added some data.

Hosts.—Bombina bombina (Linnaeus) [*Bombinator igneus* Laurenti] and *Bombina pachypa* (Bonaparte) [*Bombinator pachypus* Bonaparte], from central and southern Europe. It is also reported from *Discoglossus pictus* Otth, *Pelobates fuscus* (Laurenti), *Rana esculenta* (Linnaeus), *Uperoleia marmorata* Gray, and *Triturus vulgaris* (Linnaeus) [*Triton taeniatus* Schneider], but it would be well to scrutinize again the Opalinids from the latter six hosts to be sure they are of this species.

Measurements of a large individual.—Length of body 0.33 mm.; width of body 0.068 mm.; length of nucleus 0.0326 mm.; width of nucleus 0.01087 mm.; length of endospherule 0.003 mm.; width of endospherule 0.001 mm.; cilia line interval, anterior 0.0023 mm., middle 0.0037 mm., posterior 0.0037 mm.

Some individuals of the intermediate forms of *Protoopalina caudata* quite closely resemble *P. intestinalis* in form, lacking a definite spinelike process. But the posterior end of the body, even in these individuals, is usually more pointed, less rounded, than in *P. intestinalis. Protoopalina intestinalis* in general is somewhat more slender than *P. caudata*, except the form of the latter which we have called *attenuata* and the latter have the posterior part of the body more gradually tapering and more slender than *P. intestinalis.* In almost all infections there will be some individuals showing some nuclei in active mitosis. By counting the chromosomes in some of these which are in an anaphase condition the species can be determined confidently. *P. intestinalis* has eight macrophromosomes; *P. caudata* has six. *P. intestinalis* is the larger, but of course, in the spring, at the time of active division, the individuals of all species become small. One not very familiar with the Opalinids may find it difficult to distinguish at a glance *P. intestinalis* from *P. caudata.* Attention should be paid to the larger size, the somewhat more slender form, the rounded posterior tip of the body, and if possible to the chromosome number.

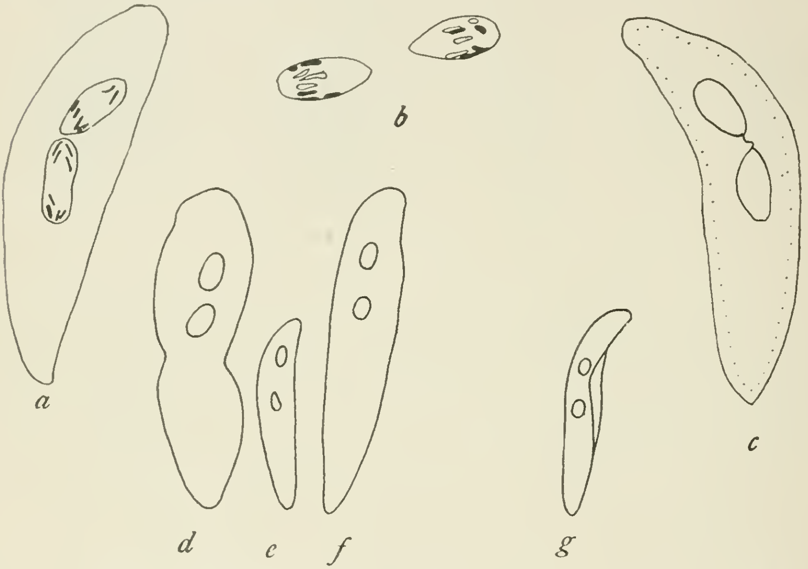

FIG. 27.—PROTOOPALINA INTESTINALIS, FROM BOMBINA BOMBINA; *a*, IN AN ANAPHASE OF MITOSIS; *b*, ANAPHASE NUCLEI FROM A DAUGHTER CELL; *c*, ENTERING UPON MITOSIS; *d*, IN TRANSVERSE FISSION; *e*, AN ORDINARY INDIVIDUAL; *f*, AN INDIVIDUAL ENLARGED PREPARATORY TO FISSION, THOUGH THE NUCLEI HAVE NOT YET ENTERED UPON MITOSIS; *g*, AN ORDINARY INDIVIDUAL FROM A DIFFERENT HOST. *a* TO *c* MAGNIFIED 460 DIAMETERS; *d* TO *g* MAGNIFIED 117 DIAMETERS.

Specimens of *P. intestinalis* have been deposited with the United States National Museum as Cat. Nos. 16441 (from *Bombina bombina*) and 16442 (from *Bombina pachypa*).

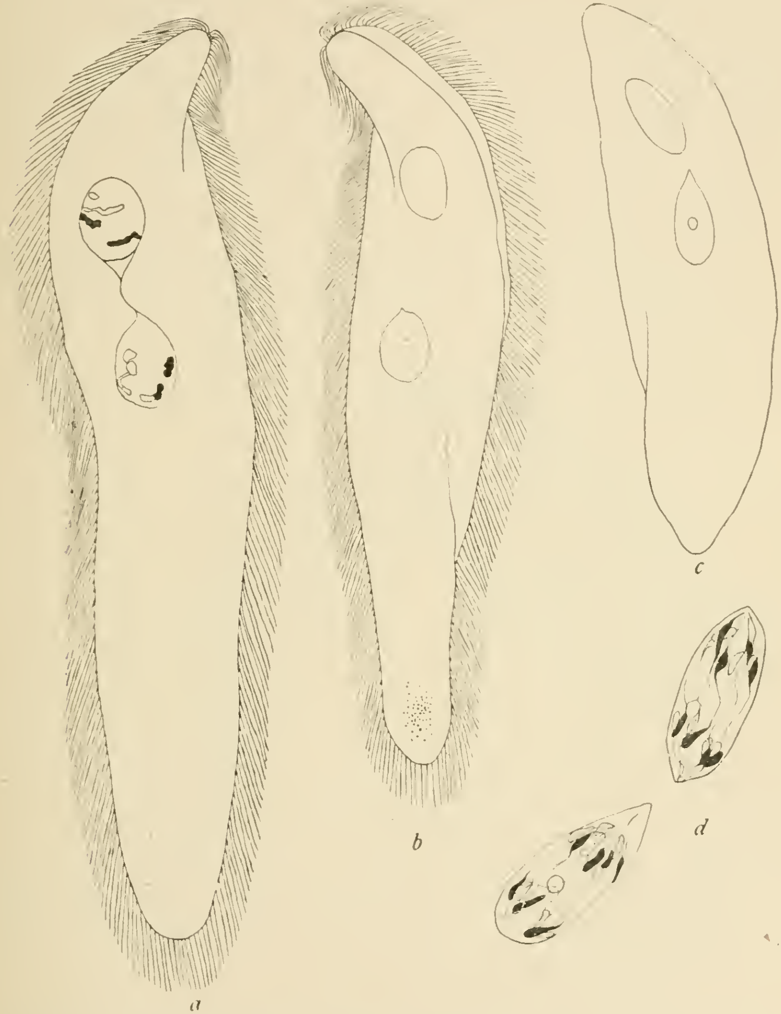

Fig. 28.—Protoopalina intestinalis, from Bombina pachypa: *a* to *c*, magnified 307 diameters; *d*, the two nuclei from the individual shown in *c*, each nucleus in an anaphase of mitosis. Only the macrochromosomes and some of their fibers are shown. The circle in the lower nucleus represents the nucleolus. Fig. *d*, magnified 667 diameters. (After Metcalf, 1909.)

PROTOOPALINA ADELAIDENSIS, new species.

Type.—United States National Museum Cat. No. 16624.

Host.—*Hyla adelaidensis* Gray, United States National Museum specimen No. 59951, from Margaret River, northwestern Australia, received from the Museum and Art Gallery of Western Australia.

Measurements: A of a rather small individual; B of a large individual—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.109	0.135
Width of body	.0271	.04
Diameter of nucleus	.0075	.0098
Diameter of endospherule	.002	.002

The cilia are long.

This *Protoopalina* has something the form of *P. intestinalis*, but is in general broader in proportion to its length. It is very much smaller and has very much smaller nuclei. The nuclei in most individuals are spherical and in a reticulate condition. A few of the smaller individuals show early metaphase nuclei, proximally pointed and their pointed ends united by a very delicate thread. The species is distinct.

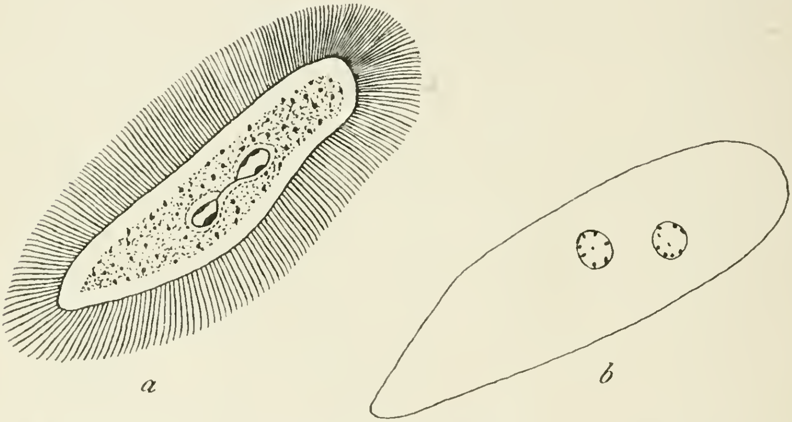

Fig. 29.—Protoopalina adelaidensis, × 460 diameters: *a*, a daughter cell; *b*, an ordinary individual.

PROTOOPALINA MONTANA, new species.

Type.—United States National Museum Cat. No. 16443.

Host.—*Megalophrys montana* Wagler, from Java. Five individuals were opened. One infection was found, in United States National Museum specimen No. 38955, 63 mm. long, from Tiibodas, Java, 4,500 feet altitude, no date; T. Barbour, collector(?). *Megalophrys montana* has a huge rectum, very long, with the caecal region well up under the right arm. In opening museum specimens care should be taken not to injure the posterior cartilege of the sternum.

Measurements.	Larger individual.	Smaller individual.
	mm.	*mm.*
Length of body	0.135	0.124
Width of body	.03	.0326
Length of nucleus	.0193	.02
Width of nucleus	.0097	.00109
Diameter of endospherule	.0009	.0011
Cilia line interval:		
Anterior		.00215
Posterior		.0037

The number of macrochromosomes is four.

Protoopalina montana is a little broader in the posterior third of the body than is the usual *P. intestinalis* and is more broadly rounded at the posterior extremity. It is a much smaller species, if we may judge from the one infection studied. The nuclei are relatively larger, are pear-shaped and connected by a thread, and usually each will be found in a late anaphase condition, each daughter nucleus simulating an equatorial plate. Out of fifty-two individuals observed on one slide, forty-four showed the nuclei in this condition; six had the two nuclei farther apart, but still connected by a thread, and each showing either eight irregularly arranged chromosome masses or eight massive chromosomes in two early anaphase groups of four each; one had its nucleus in a very much elongated and greatly constricted dumbbell condition, with four massive chromosomes in each of the developing daughter nuclei (fig. 30, *a*). The two daughter moieties were so far apart that one could almost as well call them distinct nuclei connected by a thread, the thread in this case, how-

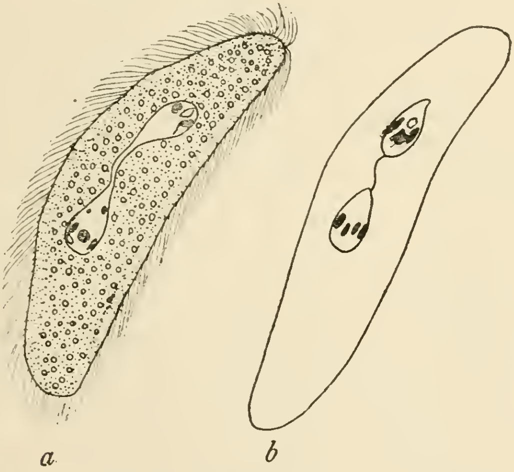

FIG. 30.—PROTOOPALINA MONTANA, × 460 DIAMETERS.

ever, having distinct walls and a clear lumen. The characteristic condition is, however, that shown in figure 30, *b*.

PROTOOPALINA HYLARUM (Raff).

Opalina hylarum Raff (1911).

Host.—*Hyla aurea* (Lesson), from Australia.

Raff's original description is copied in full:

> *Opalina hylarum*, n. sp., occurs in *Hyla aurea* only, and is distinguished from all the other binucleated forms which are circular in cross section by its enormous size. It measures on an average about 420 μ, but some individuals measuring as much as 572 μ have been met with. The average breadth is 70 μ. The body is elongatedly oval, with a rounded anterior end and a slightly rounded posterior extremity—*i. e.*, it does not taper to a point posteriorly. The protoplasm is granular, and ectosarc and endosarc are clearly distinguishable right to the posterior end [not an unusual feature, true of nearly all Opalinids]. A very well-marked feature of this species is the position of the nuclei, for they are placed far apart, the hinder one being in the posterior half of the body. The chromatin material is gathered into masses arranged around the periphery of the nucleus. This is well shown in the transverse section represented in fig. 11 [omitted here]. The body is ciliated round its entire surface, the cilia at the anterior end being slightly longer than those towards the posterior end, but there is no portion devoid of cilia.
>
> Some individuals showed only a single nucleus, in different stages of division, but these are the results of recent longitudinal division. In figures 12 to 14 [omitted here] the outlines of three specimens are shown with the positions of the nuclei indicated. In figures 12 and 13 [omitted here] the daughter nuclei have not yet separated, while in figure 14 [omitted here] division of the nucleus is completed and the nuclei have taken up their adult position.
>
> In the posterior portion of the body excretory organs are present in the form of a great number of vacuoles, forming quite a network and extending from about the middle of the body to the posterior extremity.

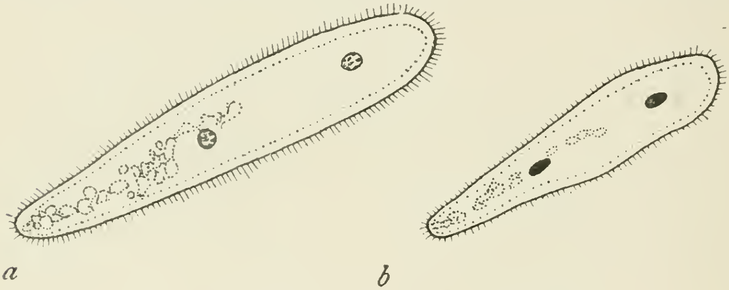

Fig. 31.—Protoopalina hylarum. (After Raff.)

This is beyond question a distinct species.

PROTOOPALINA FILIFORMIS, new species.

Type.—United States National Museum Cat. No. 16444, also paratype No. 16445.

Host.—*Rana tigerina* Daudin, two infections, from United States National Museum specimen No. 38219, 69 mm. long, the type infection, from Kagi, Formosa, September 1 to 20, 1907, H. Sauter, collector; and specimen No. 35297, 42 mm. long, same label.

Measurements of a full-sized individual.—Length of body 0.3 mm.; width of body 0.035 mm.; legnth of nucleus of one individual 0.025 mm., of a second individual 0.015 mm.; width of nucleus of first individual 0.0065 mm., of second individual 0.0075 mm.; diameter of endospherule 0.0015 mm.; cilia line interval, anterior 0.00135 mm., posterior 0.0025 mm.

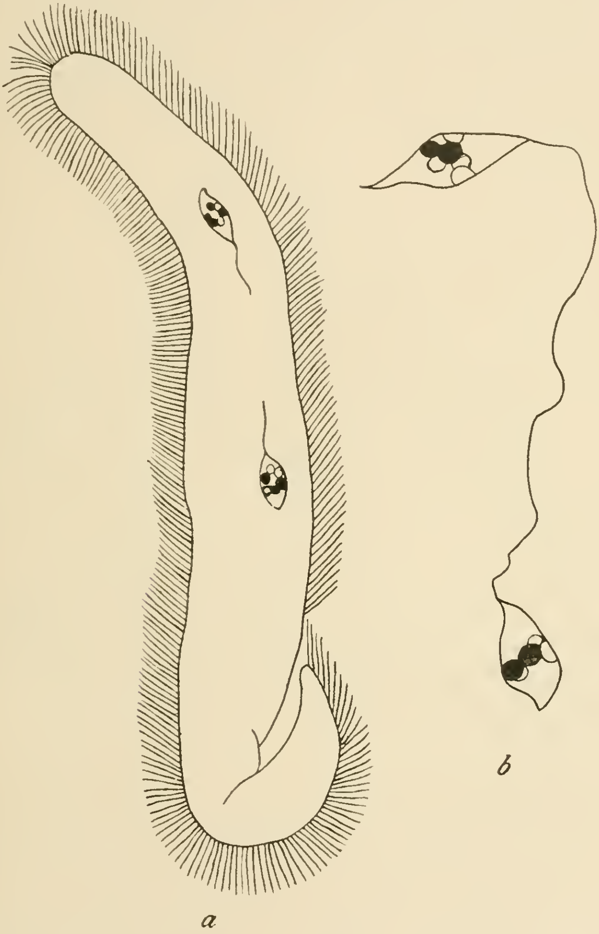

Fig. 32.—Protoopalina filiformis: *a*, an ordinary individual, × 460 diameters; *b*, the nuclei of a similar individual. × 1,000 diameters.

In all individuals of this species, except of course those in active mitosis, only one of which was found, the nuclei are in the condition of two pear-shaped or spindle-shaped daughter nuclei, still united by a very long thread. Each daughter nucleus has passed a little beyond the anaphase, but still shows apparently six macrochromosomes somewhat irregularly arranged, evidently beginning to pass into the metaphase condition. The form of the body is shown in the figure. Its enlongated slender form suggests the name given.

PROTOOPALINA TENUIS (Raff).

Opalina tenuis RAFF (1912).

Hosts.—Crinia signifera Girard, two individuals from Australia, reported by Raff, and two individuals from Australia, in the United States National Museum, Nos. 26411 (19 mm. long), and 26412 (16 mm. long), scant infection, both hosts collected by J. D. Ogilby; also *Uperoleia marmorata* Gray [*Hyperolius marmoratus* Rapp], from Narbethong, Australia, reported by Raff.

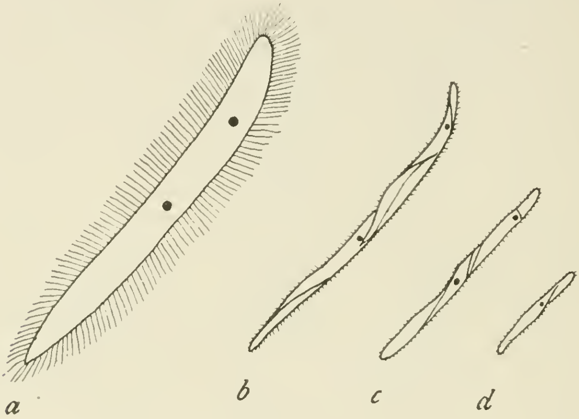

FIG. 33.—PROTOOPALINA TENUIS. (After Raff.)

Raff's description in full, is as follows:

> *Opalina tenuis*, new species. This binucleated form was found in the large intestine of two specimens of the brown froglet, *Crinia signifera*, one coming from Narbethong, near Healesville, the other from Mentone, for both of which my thanks are due to Miss O. B. Davies, B. Sc. I have also found it in another small frog from Narbethong, which I take to be *Hyperolia marmorata* [*Uperoleia marmorata* Gray]. They were present in very large numbers, and measured on an average 530µ long and 36µ broad. They present a very attenuated or drawn-out appearance, and exhibit the usual corkscrewlike motion. The cilia are very long and of much the same length all over the body, but at the anterior end they are much more numerous and thickly arranged. The anterior nucleus is situated very near the front end of the body, while the posterior one lies in about the middle of its length, or slightly in the posterior half. In the younger and shorter forms the position of the anterior nucleus varies, the individual represented in Fig. [33, *a*] having it situated further down the body than is usual in the larger forms. This individual measured 215µ in length. The smallest forms measured 100µ in length. *O. tenuis* oc-

curred along with *O. intestinalis* in *Hyperolia marmorata*, but in *Crinia signifera* it was the only species of *Opalina* present.

As noted above, I have had for study two infections of *Protoopalina tenuis* from Australian *Crinia signifera.* The measurements of a full-sized individual are:—Length of body 0.2+ mm. (too much coiled to measure accurately); width of body 0.011 mm.; length of nucleus, first specimen 0.018 mm., second specimen 0.0195 mm., third specimen 0.024 mm.; width of nucleus, first specimen 0.0068 mm., second specimen 0.00975 mm., third specimen 0.012 mm.; diameter of endospherule 0.002 mm.; cilia line interval, anterior 0.0015 mm., posterior 0.00333 mm.

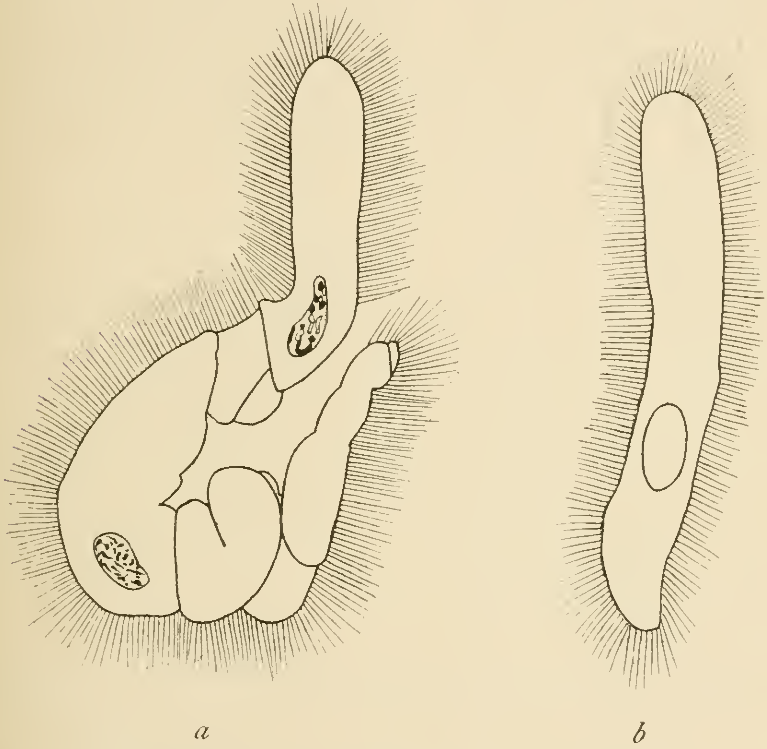

Fig. 34.—Protoopalina tenuis: *a*, an ordinary individual; *b*, an anterior daughter cell after transverse fission. × 460 diameters.

The nuclei in most individuals are ellipsoidal and in almost a reticulate condition with about 16 small chromatin masses. This is not enough to determine that the number of massive chromosomes is eight, but it indicates the probability of that number. In a few instances nuclei apparently more spheriodal were seen, but the bodies of these animals were much coiled and contorted and it may be that the nuclei were seen partly in polar view. In one instance, only, two pear-shaped nuclei were seen lying far apart in the body and connected by a very long thread. No nuclei were found in an anaphase of mitosis, so that there was no favorable opportunity for determining the chomosome number. Raff figures spherical nuclei, but her figures are small and may not be intended to show detail.

This species is very similar to *P. filiformis*, but its nuclei, except of course those in active division, are resting in a much later stage of mitosia, each of the two being elongated preparatory to entering on the next mitosis, while in *P. filiformis* the last mitosis is not yet completed, the still united daughter nuclei being each in an anaphase stage. The two species seem clearly distinct because of this difference in the mitotic phase of their resting nuclei, and because of the difference in the number of massive chromosomes.

Specimens of *P. tenuis* have been deposited with the United States National Museum as Cat. No. 16646.

PROTOOPALINA AFRICANA, new species (fig. 35, p. 61).

Type.—United States National Museum Cat. No. 16447.

Host.—*Rana crassipes* Buchholz and Peters, one infection, from United States National Museum specimen No. 48852, 55 mm. long, from Kribi, the Cameroons. This *Rana* was received from the Museum of Comparative Zoology at Cambridge, Massachusetts.

Measurements of an ordinary individual.—Length of body 0.24 mm.; width of body 0.028 mm.; length of nucleus, one specimen 0.0129 mm., second specimen 0.0102 mm.; width of nucleus, first specimen 0.00424 mm., second specimen 0.0035 mm.; length of endospherule 0.002 mm.; width of endospherule 0.0012 mm.; cilia line interval, anterior 0.00112 mm.. posterior 0.00275 mm.

This species resembles somewhat *P. tenuis* and *P. filiformis*, but is smaller than they. It bears no cilia on the posterior end of the body, which ends in a very sharp point, almost a spine. The nuclei of this species are slenderly ellipsoid or spindle-shaped. Their histological condition in my material is not such as to permit study of the mitotic condition in detail. From their form it is probable that the nuclei are in the anaphase condition. Some smaller individuals are found, probably fresh from division (fig. 35, *c*), whose nuclei are in the form of a very long slender dumb-bell, not having reached the usual resting condition for the species.

Protoopalina africana may perhaps be regarded as an intergrading form between the greatly elongated species, *P. tenuis* and *P. filiformis*, on the one hand, and the group of posteriorly slender-pointed species, including *P. xenopodos* and perhaps *P. nutti* from Africa, *P. diplocarya* from South America, and *P. acuta* and *P. papuensis* from Australasia. *Protoopalina caudata* form *attenuata* from Europe approaches this group.

PROTOOPALINA XENOPODOS, new species.

Type—United States National Museum Cat. No. 16628.

Host.—*Xenopus calcaratus* Buchholz and Peters, six infections from the Congo, Africa. The type infection is from American Museum of Natural History specimen No. 9750, 43 mm. long, female with eggs, from Medje, Belgian Congo, June 6, 1910; American Museum Congo Expedition collectors.

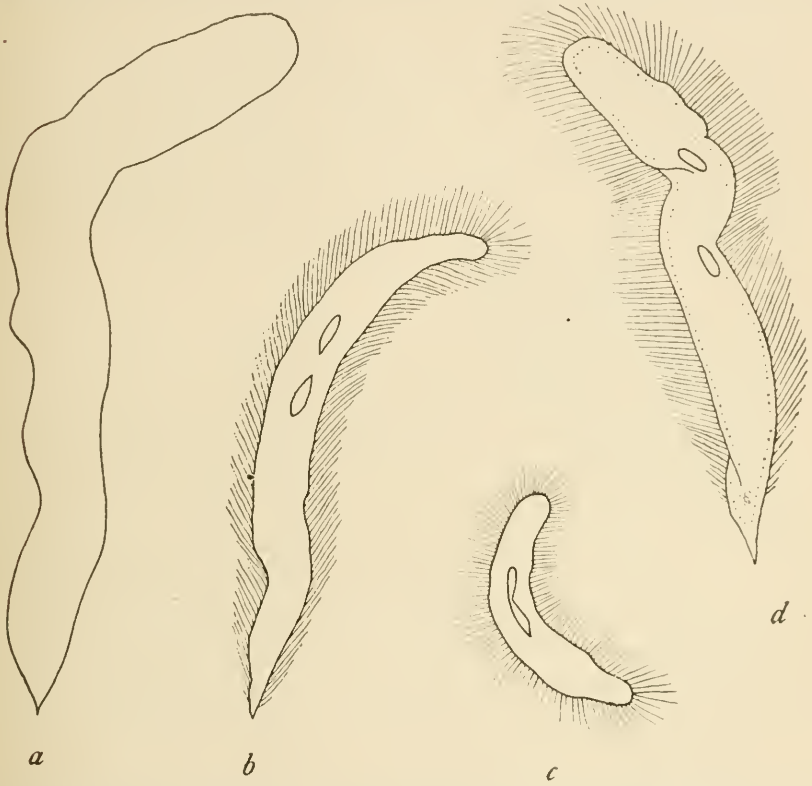

FIG. 35.—PROTOOPALINA AFRICANA, × 460 DIAMETERS: *b*, IS A CELL WHICH AFTER FISSION HAS NOT QUITE REACHED AGAIN THE USUAL CONDITION, WHICH IS SHOWN IN *d*; *c* IS A DAUGHTER CELL.

Measurements: A, of an ordinary individual from the type infection (fig. 36, b); B, of an elongated, slender individual (fig. 36, c); C, of shorter forms characteristic of one infection (fig. 36, d)—

Measurements.	*A*	*B*	*C*
	mm.	*mm.*	*mm.*
Length of body	0.1345	0.218	0.1
Width of body	.0253	.03	.0264
Length of posterior process	.012	.031	.0287
Length of nucleus	.012	.0195	.0126
Width of nucleus	.0086	.006	.0069

The specimens of *Xenopus* were all preserved in formalin and in consequence the Opalinids are not in good condition for detailed study. The lines of insertion of the cilia can hardly be seen, and the endospherules can hardly be measured. The details of nuclear structure are not so clear as to allow determining with confidence the number of the chromosomes, but the general nuclear condition can be observed.

This *Protoopalina* belongs to the group of species which have the posterior end of the body prolonged into a tapering, slender, pointed process, a group of species including *P. diplocarya* from Chile, and *P. acuta* and *P. papuensis* from Australasia, and should have been described in connection with them. The considerable resemblance to *P. africana* led to describing it here, but the other arrangement would perhaps be better.

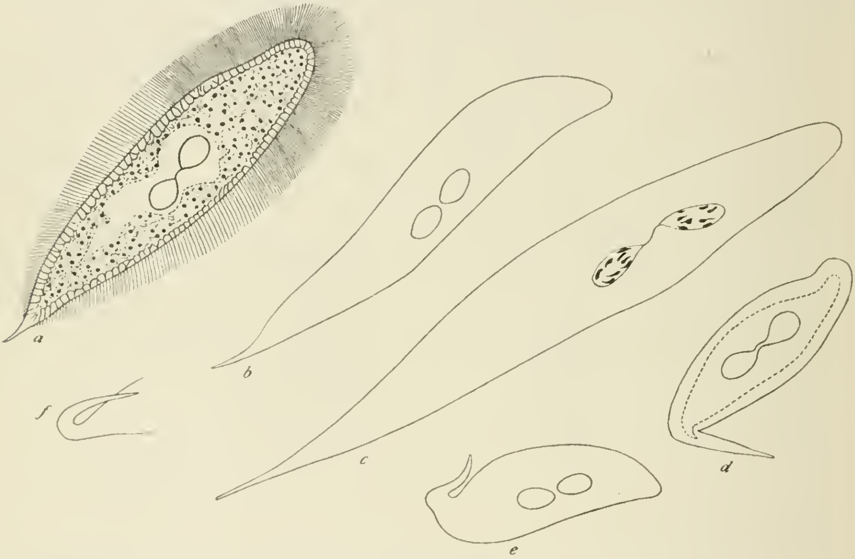

Fig. 36.—Protoopalina xenopodos, × 460 diameters: c shows nuclei in an early telophase of mitosis.

In the usual mitotic condition of its nuclei *Protoopalina xenopodos* differs from all the other pointed species of the genus. Exceptional individuals, with nuclei in active division (fig. 36, *c*), show chromatin masses (apparently eight in number ?) in place upon the spindle. The ordinary nuclei, not in active mitosis, show the same (eight ?) distinct chromatin masses, but not in any definite arrangement. These nuclei, in the usual condition, are elongated in the axis of the body, being either ellipsoid, or frequently somewhat pear-shaped. The two nuclei are found to be connected by a thread in case the condition of the specimen is favorable for observation. Probably they are always so connected, except in dividing cells. The eight (?) chromatin masses probably indicate four as the true macrochromosome number, for in nuclei in the anaphase stage (fig. 36, *c*) there are two groups of four (?) macrochromosomes each in each elongated nucleus. It is, however, unsafe to accept definitely counts of chromosomes in nuclei whose preservation is as poor as in these specimens, which were preserved in formalin.

PROTOOPALINA SATURNALIS (Leger and Duboscq).

Opalina saturnalis LEGER and DUBOSCQ (1904).

Specimens have been deposited with the United States National Museum, being Cat. No. 16459.

Host.—The marine fish *Box boops* Linnaeus, from the Mediterranean Sea. This is the only non-Amphibian species known to contain Opalinids.

Protoopalina saturnalis was discovered and carefully described by Leger and Duboscq in 1904. Several of their excellent figures are here copied. As I have had only moderately well preserved material, and no living animals, for study, the description here given is taken mostly from Leger and Duboscq's much fuller description.

Measurements of an average individual of the elongated form, from my material.—Length of body 0.152 mm. (Leger and Duboscq report individuals up to 0.25 mm. in length); width of body 0.022 mm.; length of posterior "spine" 0.00833 mm. to 0.019 mm.; length of anterior cilia 0.02 mm. to 0.025 mm.; length of resting nucleus 0.0112 mm.; width of resting nucleus 0.0092 mm.; length of endospherules 0.008 mm.; width of endospherules 0.0033 mm.; cilia line interval, anterior 0.0022 mm., posterior 0.0052 mm.

Measurements of an ordinary individual of the ovoid form, after Leger and Duboscq.—Length of body 0.1 mm.; width of body 0.06 mm.; length of posterior spine 0.005 mm.; length of anterior cilia 0.01 mm. (from Leger and Duboscq's text-figure 3); diameter of resting nucleus 0.0108 mm.; length of anaphase nucleus 0.0233 mm.; width of anaphase nucleus 0.00833 mm.

All the *Box boops* at Cavalière and Cannes are said to be infected, the intestine and the rectum showing very numerous *P. saturnalis*. At Banyuls three out of five of the young *Box boops* contain the Opalinids. *Box salpa* Linnaeus, which lives in the same regions, never contains the Opalinids.

Protoopalina saturnalis is pale yellow, the color being due to the presence in the ectosarc alveoles of a substance which looks like, but is not in reality, oil. [Many species of Opalinids have a similar yellow color from the same cause. See Metcalf (1909).] This species, like *Protoopalina caudata*, *P. orientalis*, *Cepedea dimidiata*, and some

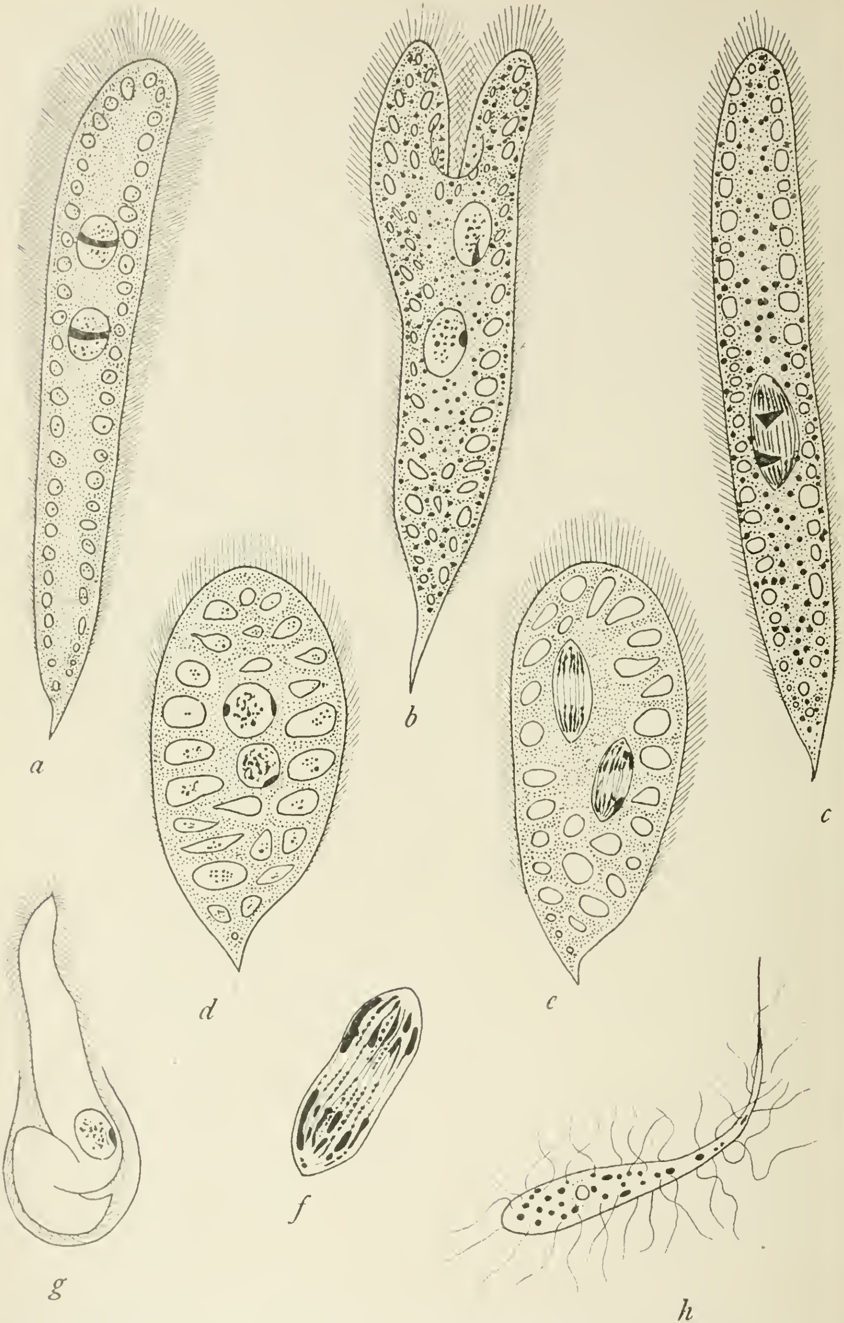

FIG. 37.—PROTOOPALINA SATURNALIS; *a*, AN ORDINARY INDIVIDUAL; *b*, AN INDIVIDUAL IN LONGITUDINAL FISSION; *c*, A DAUGHTER CELL FROM LONGITUDINAL FISSION; *d* AND *e*, INDIVIDUALS OF THE STOCKY FORM, THE LATTER PREPARING FOR FISSION; *f*, AN ANAPHASE NUCLEUS; *g*, AN INDIVIDUAL LEAVING THE CYST; *h*, A MICROGAMETE; *f*, IS MAGNIFIED 1,500 DIAMETERS, THE OTHER FIGURES 600 DIAMETERS. (After Leger and Duboscq.)

other species, has two sorts of individuals; those of an elongated form, figure 37, *a* to *c*, and others which are ovoid, figure 37, *d* and *e*. Besides these there are many intermediate individuals. Some of the large individuals seem to arise from ordinary elongated forms by increase in thickness. Leger and Duboscq write:

> The elongated forms result from longitudinal division, while the massive forms are produced by transverse fission.

Study of conditions in *P. caudata*, *Cepedea dimidiata*, and other species with both slender and stocky forms, causes me to doubt if the matter is quite so simple as this, and the doubt is emphasized by study of Leger and Duboscq's figures. In their figures of the ovoid forms the ectosarc is enormously thickened and contains large inclusions of the pseudo-oily nature, while in the elongated forms the ectosarc is much thinner and its inclusions smaller. I am more inclined to suspect that the ovoid form may be associated with peculiar nuclear physiological phenomena rather than with the manner of division. I have found some of the stocky forms of *P. caudata* with greatly enlarged nuclei. I am, however, unable to suggest the nature of the nuclear condition which may be associated with the thickening of the body.

The cilia are longest anteriorly, diminishing in length posteriorly, the posterior portion of the body being naked. Leger and Duboscq say that there are about 20 longitudinal spiral lines of cilia upon an ordinary elongated individual of average size, and that the distance between them is constant—about 0.003 mm. This statement that the intervals between the lines of insertion of the cilia are uniform is of interest. In nearly all species of all four genera of Opalinids which I have studied the cilia line interspaces in the anterior end of the body are but one-half, or one-third, or even in one species but one-fourth as great as they are posteriorly. According to Leger and Duboscq, in *P. saturnalis* the cilia lines are no more closely set anteriorly than they are posteriorly.

Leger and Duboscq describe a single minute individual (fig. 37, *h*), which, from its form and its long sparse cilia, is either a microgamete or a microgamete mother cell (see Metcalf, 1909). It needs but a ball of sticky protoplasm near its posterior tip to be a typical microgamete, even to the right angle bend at the base of the "tail." This individual is of great interest. It indicates that fertilization probably takes place in the intestine of the same individual hosts which bear the full-sized Opalinids. It is of interest, too, to note that encysted forms and individuals hatching from the cyst (fig. 37, *g*) are also found in these same adult hosts. It looks as if all the life history of the parasite occurred within the intestine of the adult *Box boops*, so that there is not, in the case of this host, a restriction of the sexual phases of the Opalinid to the young forms of the host. This might seem to lend some support to Leger and Duboscq's view that *P. saturnalis*, because of its habitat in a marine fish, is probably a more archaic form than the other species of *Opalinidae*. But the more natural suggestion seems to be that, the Amphibia being largely terrestrial forms, their Opalinid parasites confine their infection cysts to the aquatic period of the life cycle of the host, and that the encysted forms, hatching in the rectum of the tadpole, being already small, go on with a few further divisions, forming definitive gametes, a much simpler matter at this time than it would be later when the Opalinids had again attained large size.

Leger and Dubosoq note that the ciliation is always less abundant in the ovoid forms. Certain of them are said gradually to lose their cilia and to become completely naked. Thus in the same infection we find normally motile, broad individuals and completely immobile ones. Similar sparsely ciliated or naked individuals are not known for other species of Opalinids.

Protoopalina saturnalis has unusually large ectosarc spherules, those in the ovid individuals being especially large. These are said to increase in number with the age of the Opalinid and in the ovoid forms so to enlarge the ectosarc that it almost obliterates the endosarc.

The resting condition of the nuclei in this species is a peculiar metaphase with the massive chromosomes united into a ring (fig. 37, *a*) more definite than the somewhat similar ring observed in other species, as for example *P. intestinalis*. I have not had favorable material for the study of the details of nuclear structure and mitosis. Leger and Duboscq's figures indicate the presence of both massive chromosomes and granular chromosomes (fig. 37, *f*), as in other Protoöpalinas. The massive chromosomes seem to be about 10 in number. The granular chromosomes seem to be more numerous. The authors do not describe or figure any nucleolus. It does not stain with many stains.

The nuclei in *P. saturnalis* come to rest in a telophase of mitosis, and the species should naturally have been described immediately after *P. filiformis*, since we are arranging our description of species partly with reference to the mitotic phase in which their nuclei are found, but since *P. filiformis*, *P. tenuis*, *P. africana* and *P. xenopodos* from a natural series it has seemed best to complete the description of these four species before introducing the description of *P. saturnalis*.

PROTOOPALINA OVOIDEA, new species.

Type.—United States National Museum Cat. No. 16496.

Host.—*Gastrophryne texensis* (Gerard); one very abundant infection in United States National Museum specimen No. 52296, from Brownsville, Texas; Camp, collector.

Measurements of an ordinary individual.—Length of body 0.14 mm.; width of body 0.065 mm.; thickness of body 0.026 mm.; length of nucleus 0.0142 mm.; width of nucleus 0.012 mm.; diameter of endospherule 0.0012 mm.; cilia line interval, anterior 0.0022 mm., middle 0.0028 mm., posterior 0.0037 mm.

The body of this *Protoopalina* is ovate, or obovate, in face view and considerably flattened, its thickness being about one-third of its width. The posterior end is often slightly, but definitely, pointed, and in some cases the position of the excretory pore is indicated by a funnel-shaped depression at the base of this point. The axial excretory organ is usually well developed, its relation to the nuclei being clearly seen. The two nuclei are almost, but not quite, spherical, their long axes being just noticeably the greater. Their internal structure is not clear in my material.

The considerable flattening of this species suggests a transition toward the condition of the Zelleriellas (to be described later), and this transitional condition is emphasized still more by the conditions in *Protoopalina xyster* and *Zelleriella telmatobii*, to which we will now turn.

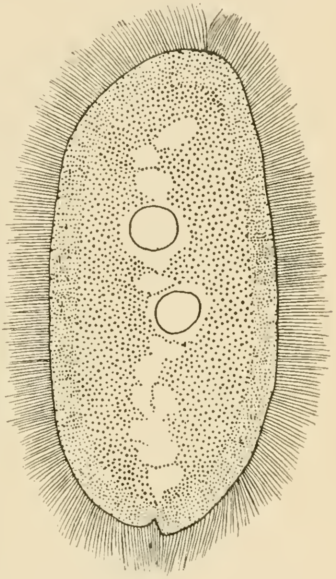

FIG. 38.—PROTOOPALINA OVOIDEA, × 460 DIAMETERS.

PROTOOPALINA XYSTER,[20] **new species.**

Type.—United States National Museum Cat. No. 16497.

Host.—*Gastrophryne usta* (Cope), one abundant infection from United States National Museum specimen No. 10021, female with eggs, from Tehuantepec, Mexico; F. Sumichrast, collector.

Measurements.—Length of body 0.197 mm.; width of body 0.093 mm.; thickness of body, anterior quarter 0.026 mm., middle 0.034 mm., posterior quarter 0.0466 mm.; length of nucleus 0.023 mm.; width of nucleus 0.0163 mm.; length of endospherule 0.00175 mm.; width of endospherule 0.0012 mm.; cilia line interval, anterior 0.0022 mm., middle 0.0028 mm., posterior 0.0037 mm.

This interesting species shows many individuals with the posterior end of the body cylindrical, while the anterior end is widened, the wider region being sharpened to an edge toward the front, like a wedge. It is shaped much like a neolithic hand adz or scraper, and this resemblance suggests the specific name. *Cepedea dimidiata* form *zelleri* has often much the same shape. The specimens in my material show a coarsely alveolated (vacuolated) endosarc and an ectosarc with much finer alveoles. The nuclei are not in good condition for study. In the resting condition they are ellipsoidal.

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[20] Greek ὁ ξυστήρ, a scraper, because of resemblance in form to a neolithic scraper.

It is suggested that the reader turn at this point to the description of *Zelleriella telmatobii*, on page 131, for comparison with *Protoopalina xyster*. The two forms are rather similar in general condition, except that in the former the flattening begun in *P. xyster* is carried to the whole of the body. Whether or not the three species, *P. ovoidea*, *P. xyster*, and *Z. telmatobii*, are actually genetically related, they at least form a series which suggests how the Zelleriellas may have been derived from the Protoöpalinas.

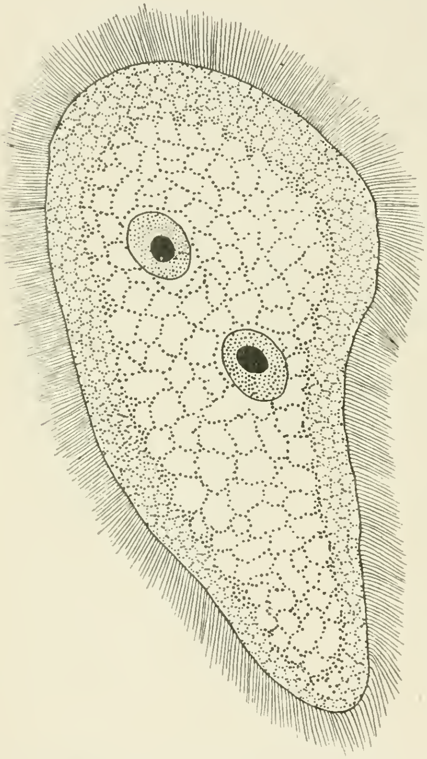

FIG. 39.—PROTOOPALINA XYSTER, × 460 DIAMETERS.

PROTOOPALINA AUSTRALIS, new species.

Type. — United States National Museum Cat. No. 16619.

Host.—Hyla aurea (Lesson), one exceedingly abundant infection in United States National Museum specimen No. 15478, from Wollongong, Illawara, New South Wales, Australia, collected January, 1840, by the United States exploring expedition. Another specimen of this Hyla from King George Sound, Australia, was opened, but no Opalinids were found.

Measurements of an ordinary individual, shown in figure 40.—Length of body, 0.125 mm.; width of body, 0.022 mm.; length of nucleus, 0.015 mm.; width of nucleus. 0.006 mm.; diameter of endospherule, 0.002 mm.; length of posterior process, 0.004 mm. to 0.007 mm. in different individuals.

This species is very different from *Protoopalina hylarum* which Raff found in *Hyla aurea*. It is but one-fourth as long, has elongated instead of spherical nuclei, and bears a long, spinelike process at the posterior end of the body. In shape of body it resembles *P. caudata discoglossi*, but its nuclei are more elongated. It is more slender than *P. macrocaudata*, and its nuclei are more elongated than those of either the latter species or *P. orientalis*. It resembles *P. regularis* (next to be described), but its nuclei, though elongated, are not yet in a definite anaphase stage, such as is found in the latter species. Its nuclei are more elongated and more pointed than those of *P. rhinodermatos*. Evidently the species is distinct.

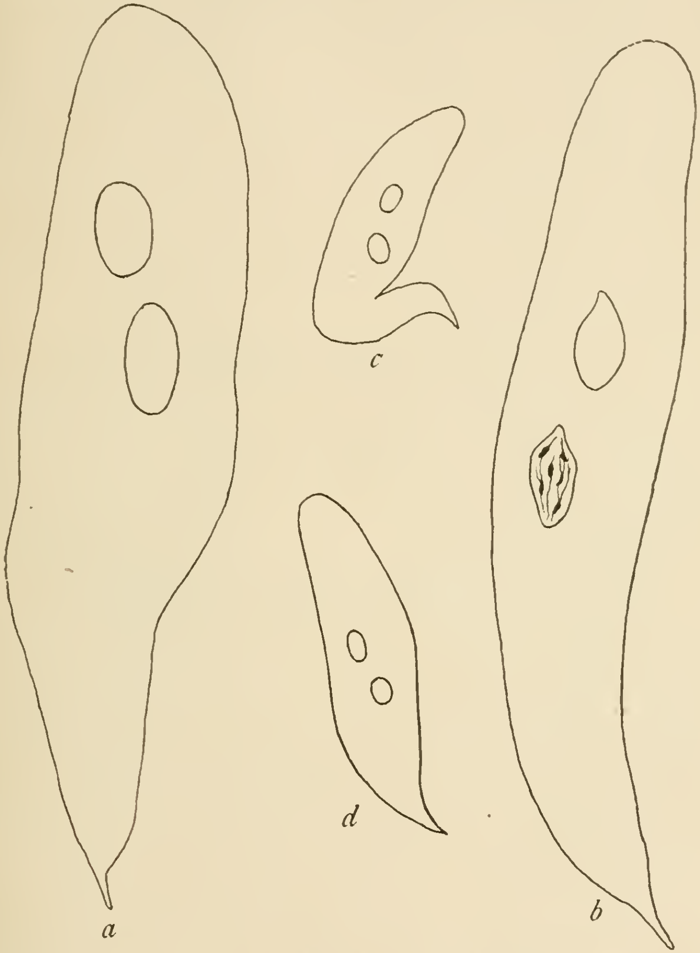

FIG. 40.—PROTOOPALINA AUSTRALIS: *a* AND *b*, INDIVIDUALS WITH NUCLEI IN ACTIVE MITOSIS PREPARATORY TO FISSION, NUCLEAR STRUCTURE NOT SHOWN IN DETAIL, × 1,000 DIAMETERS; *c* AND *d*, ORDINARY INDIVIDUALS, × 460 DIAMETERS.

The material, which has lain for 80 years in the rectum of the host, preserved in alcohol, is in good condition for general study, but the nuclear phenomena are not as clear as we would wish. The elongated, usually pointed nuclei are in a phase of mitosis approaching the equatorial plate stage. They are thus less advanced in mitosis than are the nuclei of *Protoopalina regularis*, next to be described.

PROTOOPALINA REGULARIS, new species.

Type.—United States National Museum Cat. No. 16451.

Host.—*Bufo regularis* Reuss, 4 infections out of 13 toads opened, 5 (3 infected, 2 not infected) being from the Gold Coast, western Africa, December 10 and 29, 1889, W. H. Brown, collector (one of these, United States National Museum specimen No. 16064, giving the type infection), and 8 (1 infected) from the Tana River, East Africa, November, 1892; W. A. Chanler, collector.

Measurements of the average individual shown in figure 41.—Length of body 0.196 mm.; width of body 0.0587 mm.; thickness of body 0.0375 mm.; length of posterior process 0.0087 mm.; length of nucleus 0.03 mm.; width of nucleus 0.013 mm.; diameter of endospherule 0.0025 mm.; cilia line interval, anterior 0.002 mm., posterior 0.0047 mm.

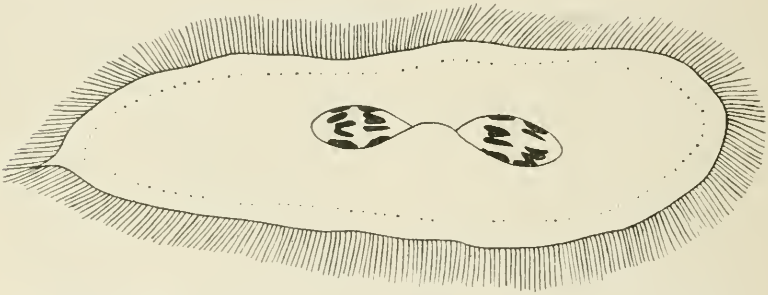

FIG. 41.—PROTOOPALINA REGULARIS, × 460 DIAMETERS. THE MACROCHROMOSOMES ARE DRAWN ONLY IN THE UPPER PORTIONS OF THE NUCLEI.

This *Protoopalina* somewhat resembles *P. caudata* in form. The posterior end of the body usually bears a distinct, naked, spinelike process which is sharp-pointed. The nuclear conditions are quite various, chiefly because rather numerous daughter cells, recently come from division, are present. The ordinary resting condition is seen in figure 41, in which we note two daughter nuclei still united by a thread. Each of these nuclei, though they are not yet completely separated, has entered upon the next mitosis and is in an anaphase condition, with two sets of distinct macrochromosomes, eight in each set. In the figure only those massive chromosomes which lie on the upper sides or the edges of the nuclei are drawn. There are in reality three other chromosomes in each set, not drawn, lying beneath those shown, making eight massive chromosomes in each set.

In addition to these, the common sort of individuals, there are others which have recently come from fission and show but a single nucleus in a dumb-bell condition, less or more constricted according to their mitotic state.

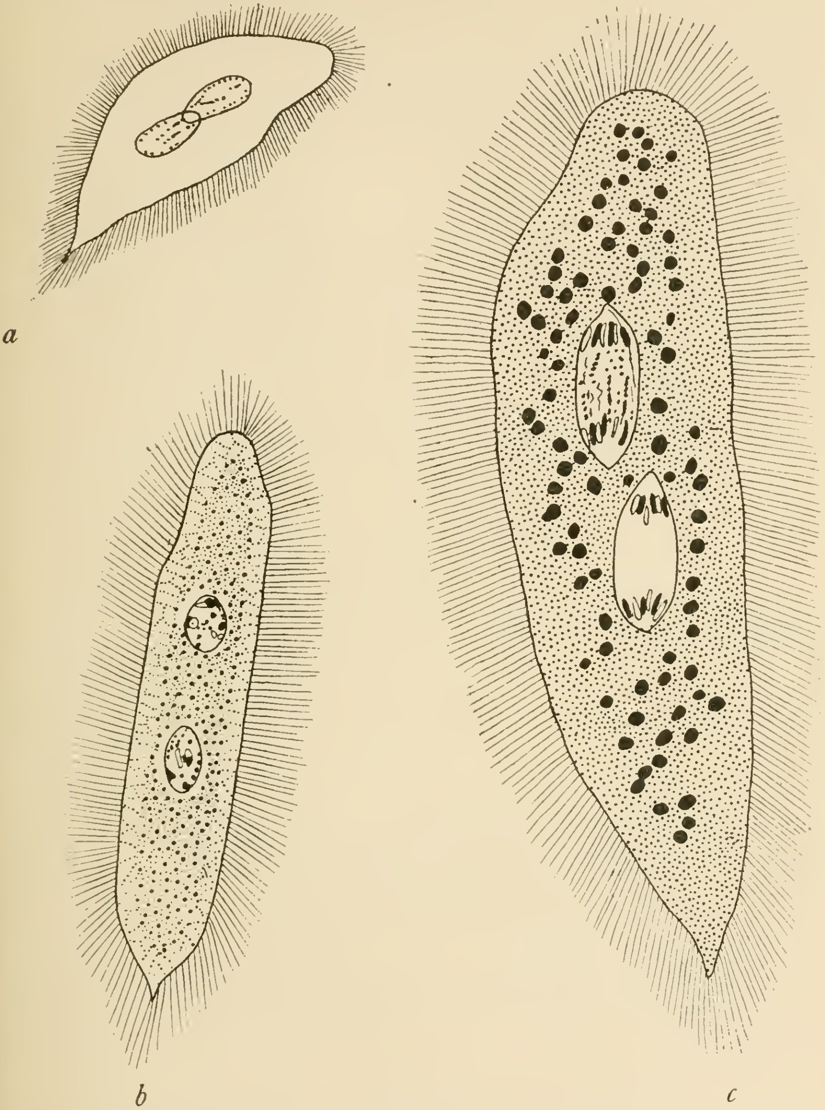

FIG. 42.—PROTOOPALINA RHINODERMATOS: *a* AND *b*, MAGNIFIED 460 DIAMETERS; *c*, THE PREVALENT SORT OF INDIVIDUAL, × 1,000 DIAMETERS.

PROTOOPALINA RHINODERMATOS,[21] new species.

Type.—United States National Museum Cat. No. 16448.

Host.—Rhinoderma darwinii Günther, one good infection from United States National Museum specimen No. 38931, 44 mm. long, from Concepcion, Chile, no date given; T. Barbour, collector. Two other individuals were opened; one contained numerous *Zelleriella darwinii;* the other contained no Opalinids.

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[21] The Greek genitive is used instead of the Latin feminine genitive, which would be awkward for this Greek neuter noun. Of course, either is allowable.

Measurements of an individual of the prevalent sort.—Length of body 0.119 mm.; width of body 0.03 mm.; thickness of body 0.0275 mm.; length of nucleus 0.022 mm.; width of nucleus 0.008 mm.; diameter of endospherule 0.00155 mm.; cilia line interval, anterior 0.00165 mm.; posterior 0.00305 mm. Massive chromosomes six in number.

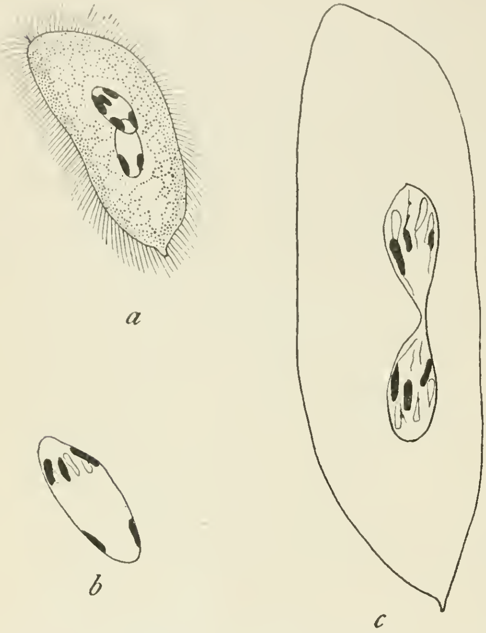

FIG. 43.—PROTOOPALINA LONGINUCLEATA: *a*, AN ORDINARY INDIVIDUAL, × 460 DIAMETERS; *b*, ITS ANTERIOR NUCLEUS, ONLY THE ANTERIOR END BEING CLEARLY SEEN OWING TO THE OVERLAPPING OF THE TWO NUCLEI, × 1,000 DIAMETERS; *c*, A DAUGHTER CELL, × 1,000 DIAMETERS.

This is a small species. Most of the individuals in my infection are of the sort shown in figure 42, *c*. The body is always sharply pointed behind, but there is no naked spinelike process. The nuclei are spindle-shaped, and in all but a few individuals are seen to be in an anaphase stage, showing clearly six massive chromosomes. Granular chromosomes can also be seen, but the preparations are not sufficiently clear to allow counting them. A few individuals are found, with oval nuclei (fig. 42, *a*) in which the chromosomes seem to be fragmented, probably passing from a reticulate stage into active mitosis. A very few others are found with nuclei as shown in figure 42, *b*. Portions of the excretory vacuole are often seen, especially around the nuclei.

PROTOOPALINA LONGINUCLEATA, new species (fig. 43, above).

Type.—United States National Musuem Cat. No. 16449.

Host.—*Telmatobius jelskii* (Peters), one infection in United States National Museum specimen No. 33864, 50 mm. long, from Guamote, Ecuador, 10,000 feet elevation, October 10, 1903. Five individuals from Ecuador and Peru were opened. One contained *P. longinucleata* and four contained *Zelleriella telmatobii.*

Measurements of two individuals: A, with two distinct nuclei; B, with single, dumb-bell shaped nucleus—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.076	0.083
Width of body	.03	.024
Length of nucleus	.017	daughter nc. .017
Width of nucleus	.006	daughter nc. .007

This little *Protoopalina* is very like the larger *P. rhinodermatos.* It has a distinct posterior process much as we see in *P. caudata.* Its ellipsoid nuclei are in an anaphase condition, showing six massive chromosomes. An occasional individual, recently come from division, is found having a dumb-bell-shaped nucleus (fig. 42, *c*). That this species is distinct from *P. rhinodermatos* is indicated by its smaller size; its definite posterior proceses; its body form, less tapering posteriorly; and its always elliptical and never spindle-shaped nuclei.

PROTOOPALINA MOSSAMBICENCIS, new species.

Type.—United States National Museum Cat. No. 16450.

Host.—*Rana adspersa* Tschudi, one scant infection from United States National Museum specimen No. 7127, 80 mm. long, female with eggs, from Mozambique, Africa, no date, W. Peters, collector.

Measurements of a full-sized individual.—Length of body 0.1065 mm.; width of body 0.0268 mm.; length of nucleus, first nucleus 0.013 mm., second nucleus 0.0185 mm., third nucleus (usual) 0.013 mm.; width of nucleus, first nucleus 0.003 mm., second nucleus 0.004 mm., third nucleus 0.007 mm.; diameter of endospherule 0.0015 mm.; cilia line interval, anterior 0.00138, middle 0.00165 mm., posterior not clearly seen.

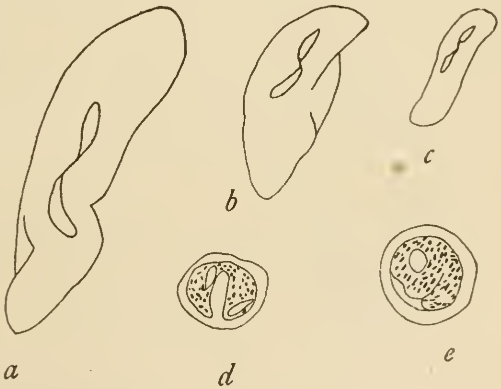

FIG. 44.—PROTOOPALINA MOSSAMBICENSIS, × 460 DIAMETERS: *d* AND *e* ARE CYSTS; IN *d* THE ANIMAL APPARENTLY IS DIVIDING WITHIN THE CYST, OR RATHER WAS APPARENTLY IN PROCESS OF DIVISION AT THE TIME OF THE ENCYSTMENT.

This very small *Protoopalina* differs from the last two species described in having the posterior end of the body rounded instead of pointed (*P. rhinodermatos*) or spinous (*P. longinucleata*). Its slender ellipsoid or spindle-shaped nuclei are usually united by a thread. Their histological condition, in my material, does not allow study of the chromosomes, but the form of the nuclei and the fact that they are still united by a thread shows them to be doubtless in an earlier mitotic phase than the nuclei of *P. rhinodermatos* and *P. longinucleata*. This species should naturally, therefore, have been described before the latter two, but it is convenient to have their nuclear condition in mind before describing this form.

PROTOOPALINA BUFONIS, new species.

Type.—United States National Museum Cat. No. 16495.

Host.—*Bufo peltocephalus* Tschudi, three infections; two, scant, from Pinar del Rio, Cuba, and another, the type infection, from United States National Museum specimen No. 51864, 127 mm. long, from Cuba, June 3, 1914; J. B. Henderson and P. Bartsch, collectors.

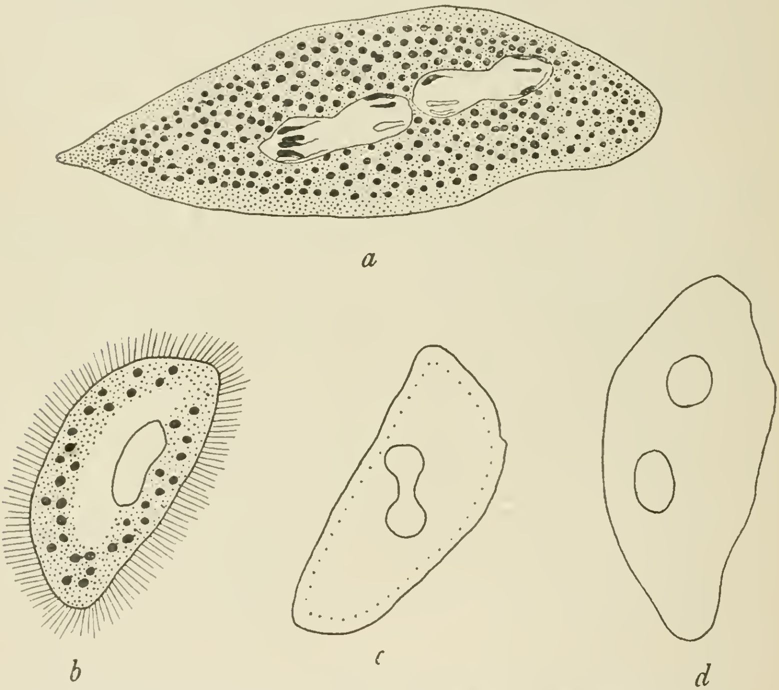

FIG. 45.—PROTOOPALINA BUFONIS, × 460 DIAMETERS: *a*, THE USUAL CONDITION; *b*, *c*, AND *d*, YOUNGER AND OLDER DAUGHTER CELLS.

Measurements: A, of an ordinary individual; B, of a large individual with nuclei in active mitosis—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.102	0.176
Width of body	.042	.0587
Thickness of body	.0155	.0152
Length of nucleus	.017	.02
Width of nucleus	.012	.007
Diameter of endospherule	.0025	.0018
Cilia line interval:		
Anterior	.0027	.00185
Posterior	.00375	.00375

This *Protoopalina* is considerably flattened, its thickness being about one-third of its width, yet its whole appearance shows it to be closely related to *P. longinucleata*, *P. rhinodermatos* and *P. regularis.* It is spindle-shaped, with broadly rounded anterior end and a narrow posterior end which may be quite slender but is never sharp pointed. Its resemblance to *P. longinucleata* parallels that of *P. intestinalis* to *P. caudata.* The daughter cells show a single dumb-bell nucleus, or the older ones show two daughter nuclei, each spherical or elongated to different degrees, as they develop toward the usual condition as shown in figure 45 *a.* Fission evidently is consummated while each of the two nuclei is dumb-bell-shaped. Each dumb-bell nucleus before fission is in an anaphase with well-defined macrochromosomes, but I find none clear enough for counting the chromosomes. I judge the number to be 8 but can not be confident. It is certainly not more than 10 or less than 6. With well-preserved material it would be very easy to determine, for there are many nuclei in the most favorable phase for observation of this point.

The considerable flatening of the body in this species suggests an approach to the condition in the Zelleriellas, soon to be described.

In this same host are a very few individuals of a species of *Zelleriella.* They are very scarce and were not observed until the stained material had faded too much for adequate study.

PROTOOPALINA SCAPHIOPODOS, new species.

Type.—United States National Museum Cat. No. 16452.

Host.—*Scaphiopus bombifrons* Cope, two infections, one (the type infection) in United States National Museum specimen No. 22265, 47 mm. long, from Los Animas, California, July 18, 1892, A. K. Fisher, collector; the other in United States National Museum specimen No. 14554, 50½ mm. long, from Black Foot Fork, Snake River, Idaho, Hayden, collector.

Measurements of an average individual.—Length of body 0.3 mm.; width of body 0.08 mm.; length of nucleus 0.042 mm.; width of nucleus 0.0125 mm.; diameter of endospherule 0.00350 mm.; cilia line interval, anterior 0.0025 mm., middle 0.0052 mm., posterior 0.0052 mm. Massive chromosomes eight in number.

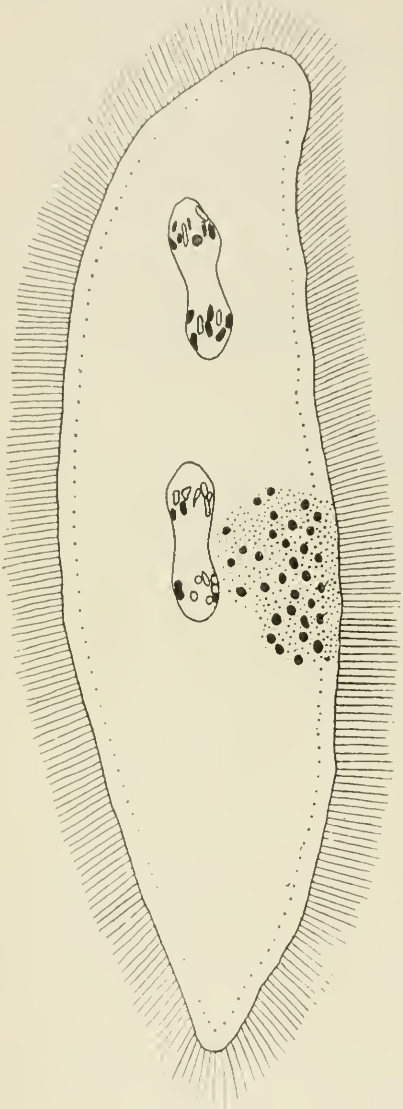

FIG. 46.—PROTOOPALINA SCAPHIOPODOS, × 460 DIAMETERS. IN THE DISTAL END OF THE ANTERIOR NUCLEUS IS A NUCLEOLUS WHICH MIGHT READILY, IN THE DRAWING, BE MISTAKEN FOR A NINTH MACROCHROMOSOME.

This is a large species much like *Protoopalina intestinalis* in form, though somewhat more stocky. The ordinary individuals have two dumb-bell shaped nuclei in the anaphase condition, showing eight massive chromosomes in each end. One individual, with nuclei of essentially the same character, was found entering upon transverse fission, and one other was found with a single nucleus in the same dumb-bell anaphase condition, a smaller individual, evidently just come from division. Thus it is indicated that fission is consummated while each of the two nuclei is dumb-bell shaped and in an anaphase stage of mitosis.

PROTOOPALINA HAMMONDII, new species.

Type.—United States National Museum Cat. No. 16453.

Host.—*Scaphiopus hammondii* Baird, five infections, all in United States National Museum material; *a*, the type infection, from specimen No. 9915, 44 mm. long, from Guanajuato, Mexico, 1877, Dugés, collector; *b*, from specimen No. 36365, 63 mm. long, from Beaver City, Utah, May 24, 1905, G. P. Engelhardt, collector; *c*, from specimen No. 52157, 25 mm. long, from Wyoming, Cooper, collector; *d*, from specimen No. 8327, 32 mm. long, from Chihuahua, Mexico, Potts, collector; and *e*, in specimen No. 52149, 19 mm. long, from Springerville, Arizona, August, 1914; J. S. Ligon, collector. Eight other toads of this species were opened; six contained no Opalinids, and two contained a flat multinucleated form, *Opalina oblanceolata.*

Measurements of an average individual.—Length of body 0.195 mm.; width of body 0.05 mm.; length of nucleus 0.037 mm.; width of nucleus 0.0113 mm.; endospherules not clearly seen; cilia line interval, anterior 0.00188 mm., middle 0.00375 mm., posterior 0.00375 mm. Six massive chromosomes.

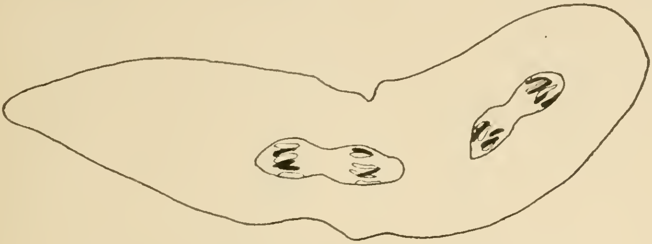

FIG. 47.—PROTOOPALINA HAMMONDII, × 460 DIAMETERS.

This species, in my infections, is a third smaller than *P. scaphiopodos*, but resembles it very closely in form. Its nuclei are closely similar, except that they have six instead of eight massive chromosomes.

PROTOOPALINA MITOTICA (Metcalf).

Opalina mitotica METCALF (1912.)

Type.—Now deposited in United States National Museum as Cat. No. 16454.

Host.—*Ambystoma tigrinum* (Green). These Opalinids were found by J. H. Powers, late in the fall, in Lincoln, Nebraska, in numerous young *Ambystoma* of this species, which had been kept in aquaria for a month or more.

Measurements.—Length of body 0.3 mm.; width of body 0.0374 mm.; length of nucleus 0.0348 mm.; width of nucleus 0.012 mm.; diameter of the disk-shaped endospherules 0.0037 mm.; thickness of same 0.001 mm.; cilia line interval, anterior 0.0025 mm., posterior 0.003 mm. Ten massive chromosomes.

This *Protoopalina* is very similar to *P. scaphiopodos* in size and form of both body and nuclei. Its massive chromosomes, however, are 10 in number. In the ordinary nuclei they are more angular and less elongated than the chromosomes of *P. scaphiopodos* or *P. hammondii*, indicating, apparently, a transition from anaphase to telophase condition. This was the first Opalinid described in which there was observed the habit of bringing the nuclei to rest in a mid-mitotic condition rather than in the reticulate condition usual in the resting nuclei of other organisms. It is this observation, and the comparative nuclear studies thus suggested, which give the key to the interpretation of speciation developed in the present paper, and it is the nuclear conditions found in these comparative studies, which give rise to the suggestions in section 6 of this paper, as to the origin of

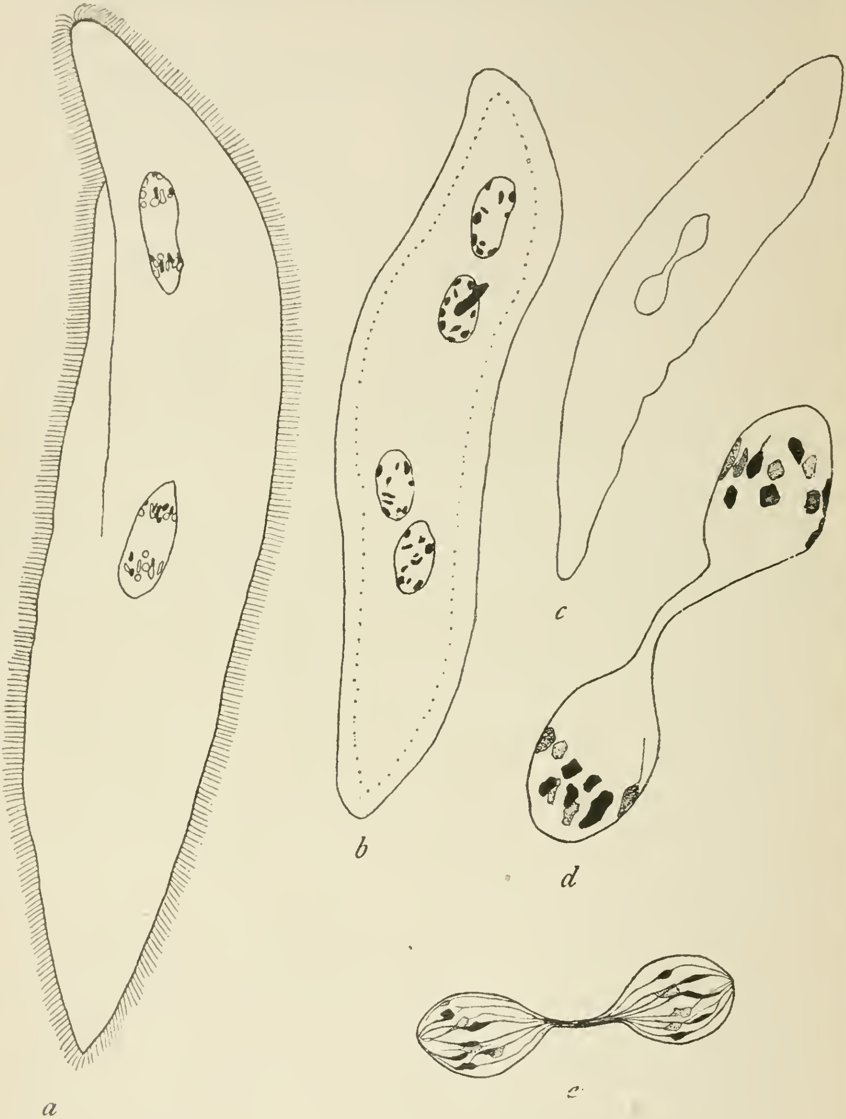

FIG. 48.—PROTOOPALINA MITOTICA: *a*, AN INDIVIDUAL OF THE USUAL SORT, × 460 DIAMETERS; *b*, A QUADRINUCLEATE INDIVIDUAL WHICH HAS, PROBABLY ABNORMALLY, DELAYED ITS FISSION, × 460 DIAMETERS; *c*, A DAUGHTER CELL, × 360 DIAMETERS; *d*, ITS NUCLEUS, × 1,485 DIAMETERS; *e*, NUCLEUS OF ANOTHER DAUGHTER CELL, WHICH SHOWS THE MACROCHROMATIN SPINDLE FIBERS. (*c*, *d*, AND *e*, AFTER METCALF, 1912.)

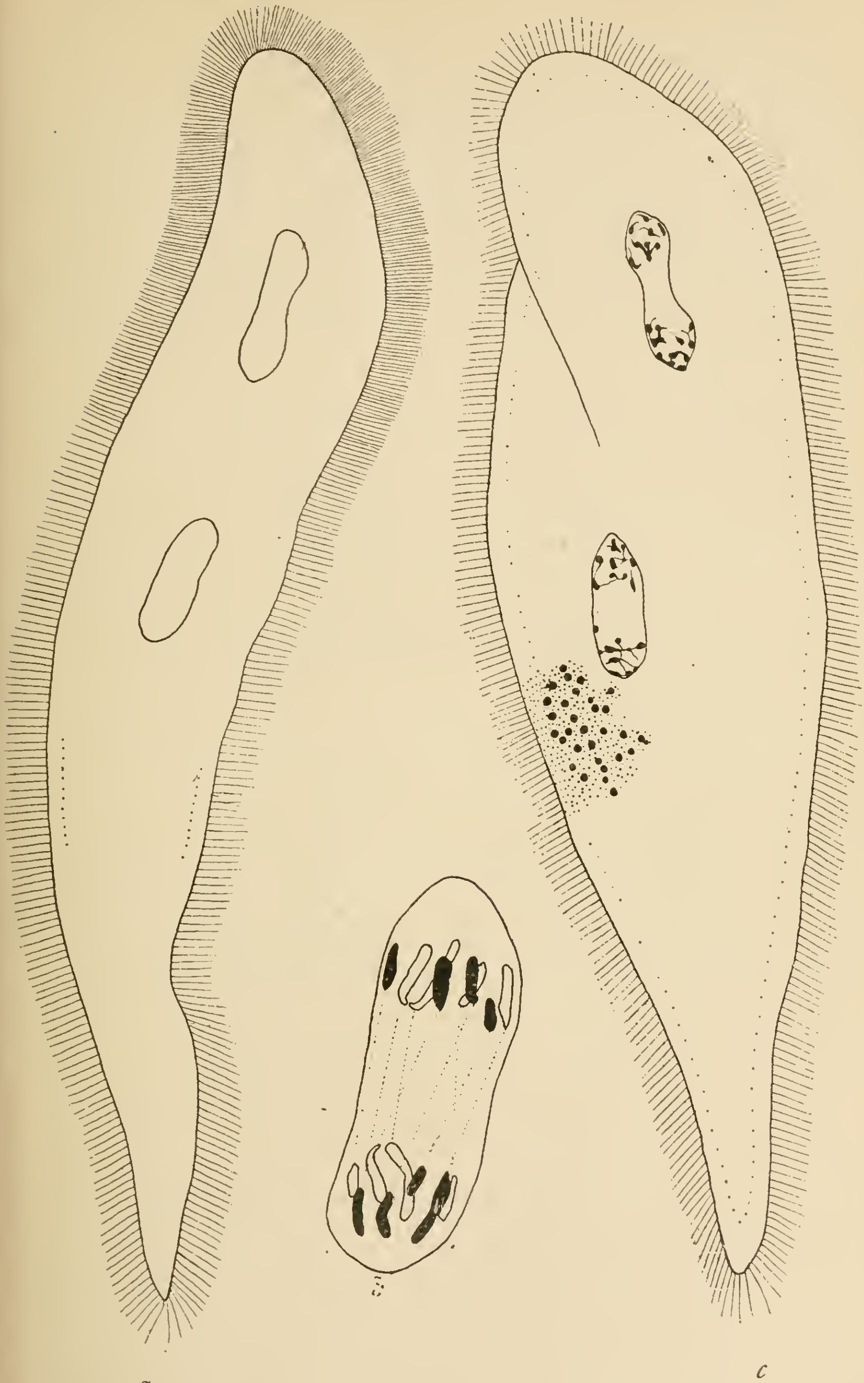

Fig. 49.—Protoopalina mexicana: *a*, an old daughter cell whose nuclei have not yet reached the telophase condition, × 460 diameters; *b*, its anterior nucleus, showing the pairs of macrochromosomes, × 2,000 diameters; *c*, an individual of the usual sort, with telophase nuclei, × 460 diameters.

the Ciliata. For fuller description of *Protoopalina mitotica* see Metcalf (1912).

PROTOOPALINA MEXICANA, new species.

Type.—United States National Museum Cat. No. 16455.

Host.—*Scaphiopus multiplicatus* Cope, one abundant infection in United States National Museum specimen No. 14599, 32 mm. long, from Mexico, September 1, A. Dugés, collector.

Measurements of an average individual.—Length of body 0.36 mm.; width of body 0.06 mm.; length of nucleus 0.0445 mm.; width of nucleus 0.013 mm.; diameter of endospherule 0.0018 mm.; cilia line interval, anterior 0.0015 mm., middle 0.00225 mm., posterior 0.000425 mm. Eight massive chromosomes.

This large *Protoopalina* is usually more slender than *P. scaphiopodos*, *P. hammondii*, or *P. mitotica*. Its nuclei have the same form as in the three species mentioned, but are in a definite telophase stage of mitosis in most individuals, as is shown in figure 49, *c*. In this figure the chromatin granules shown are only those in the upper half of the nuclei. The endospherules of *P. mexicana* are much smaller than those of *P. scaphiopodos*. In general the size of the endospherules seems to be fairly constant and of safe diagnostic value for species though in *P. bufonis* they are of considerably different sizes in different individuals.

A very few younger individuals were found (fig. 49, *a*) in which the nuclei were in an anaphase condition. In some of these the massive chromosomes were remarkably clear (fig. 49, *b*) considering the nature of the material and the manner of its preservation in the host. In this figure the pairs of chromosomes at the opposite ends of the nucleus are indicated by dotted connecting lines. That this is the true pairing is shown in the preparation not only by the form and the size of the several massive chromosomes, but also by the somewhat irregular threads of the chromatic spindle (omitted in the figure for the sake of clearness) which unite the two daughter chromosomes of each pair. One could not ask for larger and clearer nuclei for study than those some of the Protoöpalinas present.

PROTOOPALINA FORMOSAE, new species.

Type.—United States National Museum Cat. No. 16456.

Host.—*Bufo melanostictus* Schneider, 1 infection (out of 40 toads opened: 5 of the 40 toads contained other species of Opalinid), from United States National Museum specimen No. 38206, 89 mm. long, from Formosa, June 5, 1907; H. Sauter, collector.

Measurements of an average individual.—Length of body 0.12 mm.; width of body 0.02 mm.; length of nucleus 0.011 mm.; width of nucleus 0.0056 mm.; diameter of endospherule 0.00175 mm. to 0.002

mm.; cilia line interval, anterior 0.002 mm., posterior 0.0027 mm. Probably six massive chromosomes.

This rather small species is sharp pointed posteriorly, except in swollen individuals about to divide by fission, in which the posterior end is usually rounded (*b* in fig. 50). Quite divergent conditions as to mitosis are found, each of the states figured being seen.

This species seems a little more advanced in the average mitotic state of its nuclei, because of the considerable number found in the condition shown in figure 50, *a* and *b*, almost four-nucleated forms.

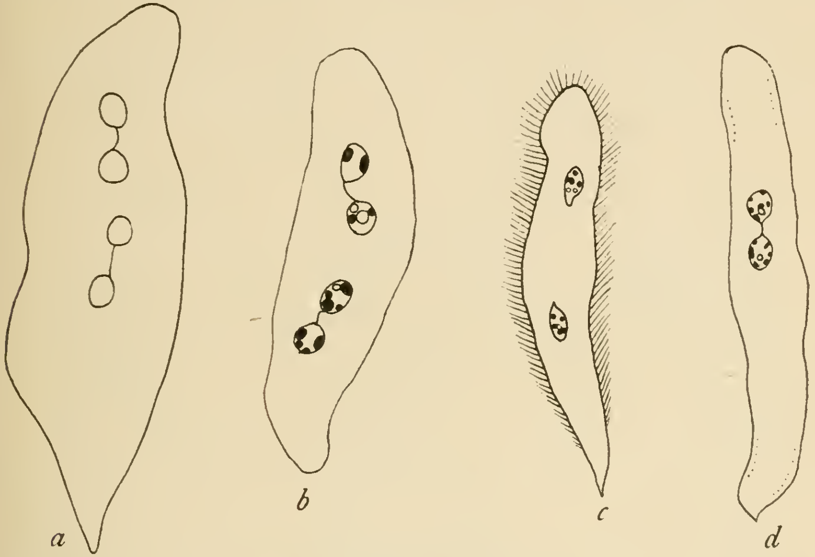

FIG. 50.—PROTOOPALINA FORMOSAE, × 460 DIAMETERS: *a* AND *b*, THE USUAL CONDITION; *c*, AN OLD DAUGHTER CELL; *d*, A YOUNG DAUGHTER CELL.

The number of massive chromosomes seems to be six. At least in a number of nuclei this is the number of chromatin masses.

PROTOOPALINA QUADRINUCLEATA, new species.

Type.—United States National Museum Cat. No. 16457.

Host.—*Rana macrodon* Tschudi, one infection in United States National Museum specimen No. 43931, 75 mm. long, from Mount Salok, Java, May, 1909; Bryant Expedition, collectors.

Measurements of an average individual.—Length of body 0.163 mm.; width of body 0.02 mm.; length of nucleus 0.012 mm.; width of nucleus 0.00652 mm.; length of endospherule 0.0015 mm.; width of endospherule 0.001 mm.; cilia line interval, anterior 0.0015 mm., posterior 0.00215 mm. Massive chromosomes probably four in number.

This small Protoopalina is distinguished by its very slender, tapering, posterior end, almost like a microgamete mother cell. The cilia are longer in front and diminish in length through the middle third of the body, being absent from the slender, tail-like posterior third. There are four distinct ellipsoid nuclei, each showing eight chromatin masses, which might indicate either eight or four as the number of the massive chromosomes. One individual was found with three nuclei (fig. 51, *c*), one being very large and in an earlier mitotic stage, almost a reticulate condition, there being still about eight large chromatin granules, while the anterior nucleus had already divided into two smaller daughters which, however, were still connected by a thread. In each of these were obscurely seen a smaller number of chromatin masses, not more than four. There therefore seem to be four true massive chromosomes. The nuclei shown in figure 51, *a*, are in about an equatorial plate stage of mitosis. (The equatorial plate in the Opalinids is never definite and regular.) Figure 51, *a*, does not attempt to show the number of the chromatin masses, not all being drawn. Figure 51, *c*, shows an individual recently come from transverse division. The anterior nucleus is already divided into two daughter nuclei. The posterior nucleus is apparently not normal, being unusually large and still undivided. The posterior end of the body has not yet taken on the regular form with a slender, elongated, naked tail.

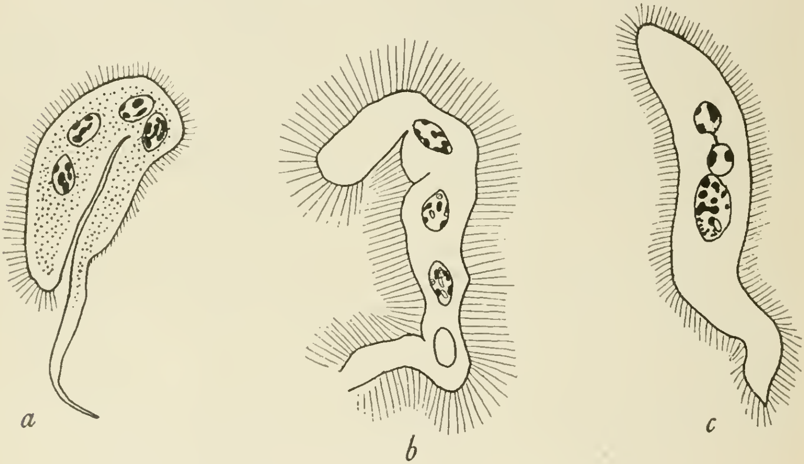

FIG. 51.—PROTOOPALINA QUADRINUCLEATA, × 460 DIAMETERS.

This is a very distinct species, not likely to be confused with any other.

PROTOOPALINA AXONUCLEATA, new species.

Type.—United States National Museum Cat. No. 16458.

Host.—*Bufo bufo asiaticus* (Steindachner), three infections in United States National Museum material. The type infection, in specimen No. 21215, 51 mm. long, and one other are from Seoul, Korea, August, 1883; P. L. Jouy collector. The third is from Southern Manchuria; A. de C. Sowerby, collector.

Measurements.—Length of body 0.23 mm.; width of body 0.03 mm.; length of nucleus 0.0156 mm.; width of nucleus 0.008 mm.; diameter of endospherules 0.0016 mm., 0.00175 mm.; cilia line interval, anterior, first specimen 0.00175 mm., second specimen 0.0022 mm., posterior, first specimen 0.003 mm., second specimen 0.0033 mm. Four massive chromosomes.

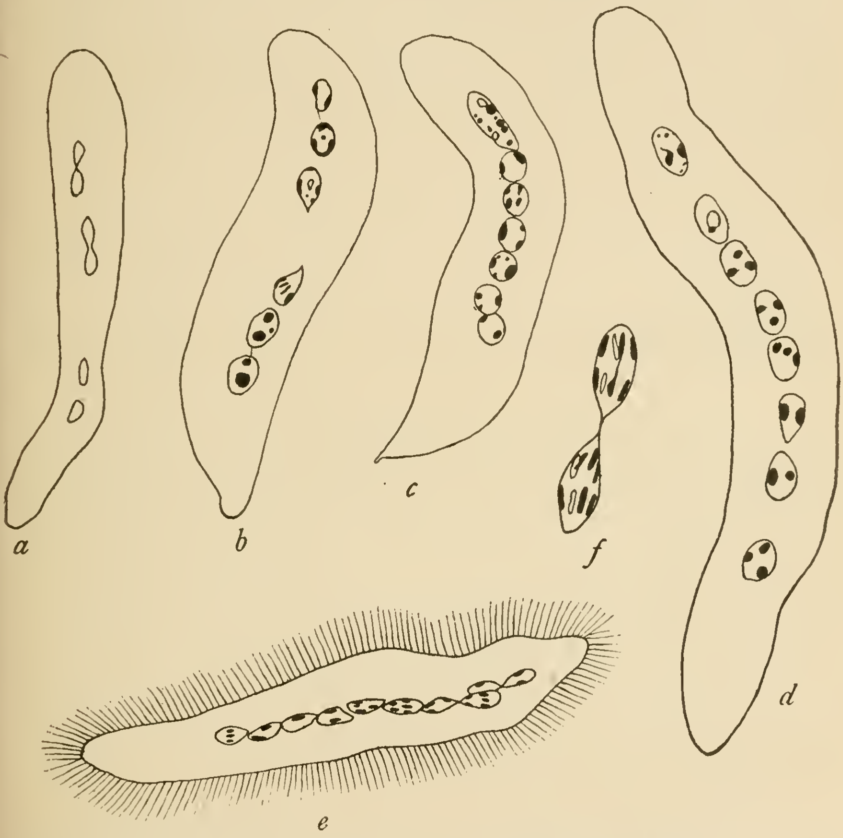

FIG. 52.—PROTOOPALINA AXONUCLEATA: *a* TO *e*, MAGNIFIED 460 DIAMETERS; *f*, THE NEXT TO THE ANTERIOR NUCLEUS OF *e*, MAGNIFIED 1,380 DIAMETERS.

This long, rather slender *Protoopalina* presents about the variety of form shown in the figures. The individuals shown in figure 52, *b* and *c* are apparently anterior daughter cells recently come from division, as indicated, first, by their pointed or irregular posterior ends, and, second, by the fact that in each case one nucleus has either not yet divided (*c*) or has not yet quite completed the restoration of the nuclear condition after mitosis (*b*). Figure 52, *e*, shows an individual whose nuclei have entered upon the next mitosis preparatory to fission. The next to the anterior pair of nuclei, though still connected by a thread as is usual, are each already in an anaphase of mitosis, showing two sets of four massive chromosomes (fig. 52, *f*).

PROTOOPALINA AXONUCLEATA LATA, new subspecies.

Type.—United States National Museum specimen Cat. No. 16622.

Host.—*Rana nigromaculata* Hallowell, United States National Museum Cat. No. 39351, from 20 miles east of Hai-shin-ssu, Shensi, China, August 11, 1909; A. de C. Sowerby, collector.

Measurements: A, of a small individual with four nuclei; B, of a large individual with eight nuclei—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.158	0.245
Width of body	.039	.051
Length of nucleus	.01	.012
Width of nucleus	.0065	.0065
Diameter of endospherule	.002	.002

This form closely resembles that found in *Bufo bufo asiaticus*, but is broader. There is in some individuals a wide, round pointed, posterior process. In both the narrow and the broad forms of this species the macrochromosome number seems to be four. In the subspecies *lata* one of these macrochromosomes is noticeably larger than

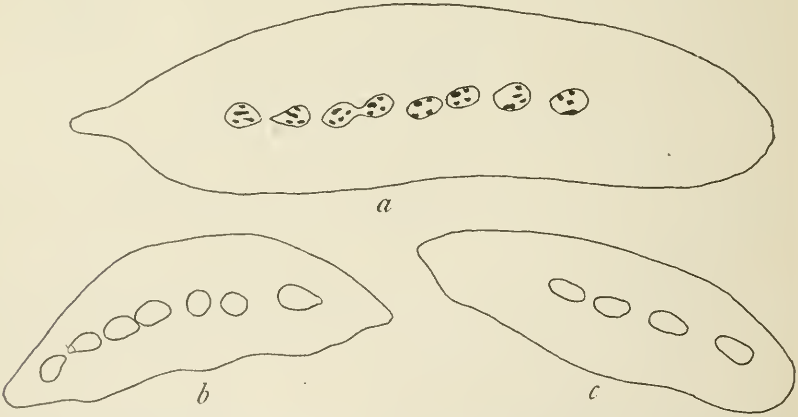

FIG. 53.—PROTOOPALINA AXONUCLEATA LATA, × 460 DIAMETERS: *a* SHOWS FOUR MACROCHROMOSOMES IN EACH NUCLEUS. IN EACH GROUP OF FOUR, ONE CHROMOSOME IS NOTICEABLY LARGER THAN THE OTHER THREE.

the other three. The nuclei of the broad subspecies are smaller than those of the species proper.

At first thought it seems that this multinucleated species and subspecies should be assigned to the genus *Cepedea*, but more careful comparison seems to show them to be multinucleated *Protoopalinae.* The nuclei are too large for *Cepedea*, and especially the small number of massive chromosomes, four, seems to place this form, beyond much doubt, among the Protoopalinas. *Protoopalina formosæ*, *P. quadrinucleata*, and *P. axonucleata* seem to form a series showing the acquirement by the most highly modified Protoopalinas of a multinucleated condition. In *P. axonucleata* the nuclei are of an even number except in cases in which one of the nuclei has delayed its division. In most individuals the nuclei are still united in pairs by connecting threads. In some other individuals the nuclei of each pair have separated. In general the daughter nuclei from the last mitosis do not separate until they enter upon the subsequent mitosis, but the separation may occur earlier.

It will be seen later that *Protoopalina axonucleata* is to be regarded as the most highly evolved of the Protoöpalinas, if my interpretation of the speciation is correct.

Genus ZELLERIELLA.

The Protoöpalinas—that is, the species of Opalinidae thus far described in this paper—are all more or less cylindrical. They are circular or broadly oval in cross section and are somewhat elongated. There is another genus, *Zelleriella*, whose species, while binucleated, are narrowly oval or flat in cross section. Their nuclei are for the most part spherical or nearly so, and they do not show in the different species quite so much diversity in mitotic condition as we observe among the Protoöpalinas described. It seems well to group all these flattened forms together under a distinct genus. This seems the more justified from the fact that we know few transitional forms between the two genera. After preliminary study of my series of Opalinids, I used the genus name *Protoopalina* to cover both the cylindrical and flattened binucleated species, and I have published this classification (Metcalf, 1918, *a* and *b*). After more detailed study, it seems both more convenient and a clearer expression of the true conditions to separate the flattened forms under a distinct genus. I am naming this genus *Zelleriella* after Zeller, whose memoir upon the reproduction and development the Opalinidae, published in 1877, is one of the best of the earlier papers on this group. Diagnostic description of this genus, like that of *Protoopalina*, is postponed until we have seen in detail the character of the several species in the family.

Taxonomic study of *Zelleriella* is difficult, for the several forms found in 49 species of Anura are very hard to distinguish. A few forms stand out as clearly distinct species. As to some of the others a definite opinion can hardly be formed. It is probable that the several species of Opalinids have divergent races, as is so usual among Euciliata. One group of forms, which might be called a species, may at both extremes of its series of races overlap other similarly divergent groups of forms. If clear-cut classification were the goal in taxonomic study, I would give up the task for the forms of *Zelleriella*, but, of course, the task is to describe conditions as they are, and this we must attempt in the present chapter upon the taxonomy. The results, however, will not be as satisfactory for *Zelleriella* as are those obtained from the study of the species of *Protoopalina*, for there will be much of doubt and much danger of confusion. This very fact of intergradation and overlapping of species is itself one of the interesting phenomena in the genus *Zelleriella* and deserves emphasis. It is one of the indications of the comparatively recent origin of this genus.

The Zelleriellas, with the exception of *Z. binucleata* (Raff) and *Z. macronucleata* (Bezzenberger) are, so far as known, confined to the Western Hemisphere—South America, Central America, the West Indies, southern and southwestern North America. Geographical segregation, therefore, gives us little clue to the present demarcation between species. As already mentioned, there is less diversity between species in the mitotic condition of the resting nuclei than there is in the genus *Protoopalina*. Size of body varies with the race and with the time of the life cycle observed. Form of body is of some help, though there is considerable diversity between the individuals of the same species. The size of the nucleus relative to that of the body differs, first, in accordance with the physiological state of the nucleus and, second, if the nuclear phenomena described by Neresheimer are normal, the nuclei vary in size according as they are primary nuclei (before the chromidia have been extruded previous to the sexual phases of the life cycle), or are secondary nuclei such as are found during the sexual period of the life history, the former being much the larger. Even if the nuclear degeneration is abnormal, as seems to be the case, it occurs, and when the conditions so established are found they are likely to prove confusing. One must, therefore, in his comparisons of species, be sure that he is comparing corresponding phases of life cycle and physiological condition in the species under consideration. The interval between the lines of insertion of the cilia is not only different in different regions of the body, as already noted for *Protoopalina*, but is different in the corresponding parts of the body in different individuals of the same species, though these differences are apparently within definite limits for each species. But the extreme interval measurements in one species may be overlapped by the extreme measurements in another species which has a different mean. Measurements of the cilia line interval do not help us much in determining species. The size and shape of the endospherules seem to be fairly constant for most species, and the size and shape differ enough with the species, in some instances, to be of some assistance. Chromosome number, when discernable, is a definite character, but the study of this feature requires well-preserved material, better preserved than most of that I have obtained from museum specimens of Anura.

It is evident that to review successfully the taxonomy of the Zelleriellas one should have data from the whole life cycle of each species. I have not any such complete material. The studies here recorded are confessedly inadequate and the conclusions reached only tentative. In numerous instances I shall make no attempt to determine definitely the species and will leave the forms unnamed, merely referring them to their hosts and leaving the naming of the forms to be done after some future study of more complete material. In such cases the organism will usually be referred to merely as the *Zelleriella* of its named host, as for instance, *Zelleriella* [of *Bufo woodhousi*], the *Zelleriella* found in Woodhouse's toad, or *Zelleriella opisthocarya* [of *Bufo typhonius*], the form of *Z. opisthocarya* found in *Bufo typhonius.* This method of designation is an awkward one, but is adopted because the terms used can not be treated by others as true taxonomic names. Their awkwardness will thus prevent future confusion. In a few instances, where there is greater probability of the species being valid, I give a tentative name, but inclose it in brackets to indicate that the name is only provisional and should not yet be recognised, as, for instance, *Zelleriella* [*engystomopsis*]. Such bracketed names are not to be taken as true taxonomic terms, but are provisional and merely a convenience for reference, to avoid circumlocution. If quoted by others the brackets also should be quoted, unless the subsequent author wishes to assume responsibility for validating the name.

ZELLERIELLA HYPOPACHEOS, new species.

Type.—United States National Museum Cat. No. 16461.

Host.—*Hypopachus variolosus* (Cope), one infection, in United States National Museum specimen No. 24830, 35 mm. long, from Guatemala; H. Hogue, collector; no date.

Measurements: A, of a large individual; B, of a small individual—

Measurements.	A	B
	mm.	*mm.*
Length of body	0.2435	0.16
Width of body	.1543	.1
Diameter of endospherule	.002	....
Length of nucleus	.0326	.0185
Width of nucleus	.023	.016

This large *Zelleriella* is quite constant in form, being of much the same shape as *Opalina ranarum.* In the smaller individuals the nuclei are nearly spherical and are often united by a thread. The largest forms show two distinct, more ellipsoidal nuclei doubtless preparing for mitosis. My material is not well enough preserved for detailed study of the nuclear phenomena, nor can one clearly see the endospherules, or the cilia and their lines of insertion.

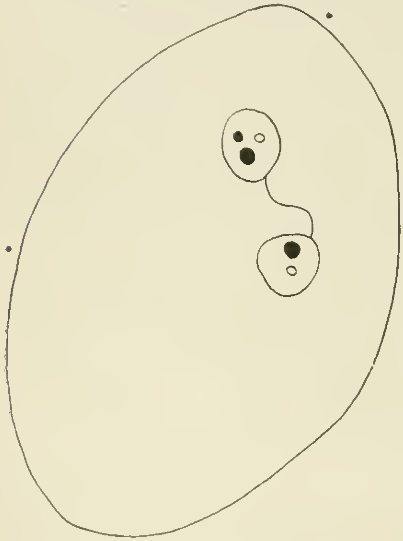

FIG. 54.—ZELLERIELLA HYPOPACHEOS, × 460 DIAMETERS. IN THIS AND IN SUBSEQUENT FIGURES THE TWO DOTS OUTSIDE THE CONTOUR OF THE BODY INDICATE THE LIMITS OF THE ANTERIOR END.

ZELLERIELLA [ATELOPODOS], new species (?).

A specimen of this form has been deposited with the United States National Museum as Cat. No. 16462.

Host.—*Atelopus varius* Stannius, one scant infection, in United States National Museum specimen No. 30633, 38 mm. long, from Pico Blanco, Costa Rica; W. M. Gabb, collector; no date.

Measurements.—Length of body 0.1575 mm.; width of body 0.1015 mm.; diameter of nucleus 0.019 mm.; diameter of endospherule not clear; cilia line interval, anterior 0.0015 mm., posterior 0.0025 mm. The number of massive chromosomes can not be determined from the material available.

This species has much the same form as *Z. hypopacheos*, but the few individuals I have show two distinct spherical nuclei, not connected by a thread as in specimens of *Z. hypopacheos* of the same size, nor ellipsoidal as are the nuclei of the larger specimens of *Z. hypopacheos.* This different mitotic condition of the nuclei suggests that *Z.* [*atelopodos*] is a distinct species, but I prefer not to name it definitely

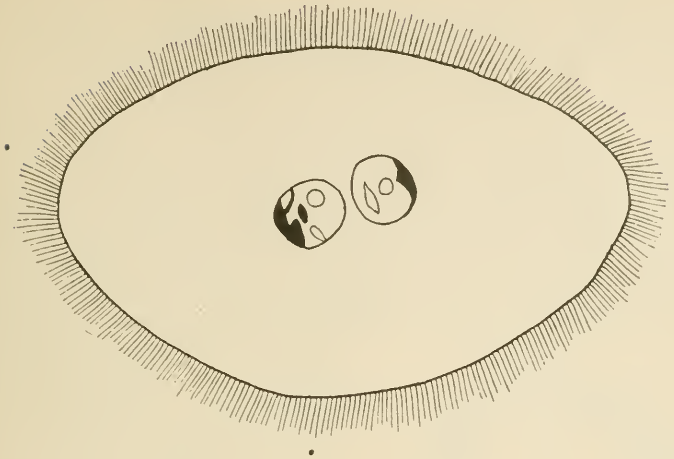

FIG. 55.—ZELLERIELLA [ATELOPODOS], × 460 DIAMETERS.

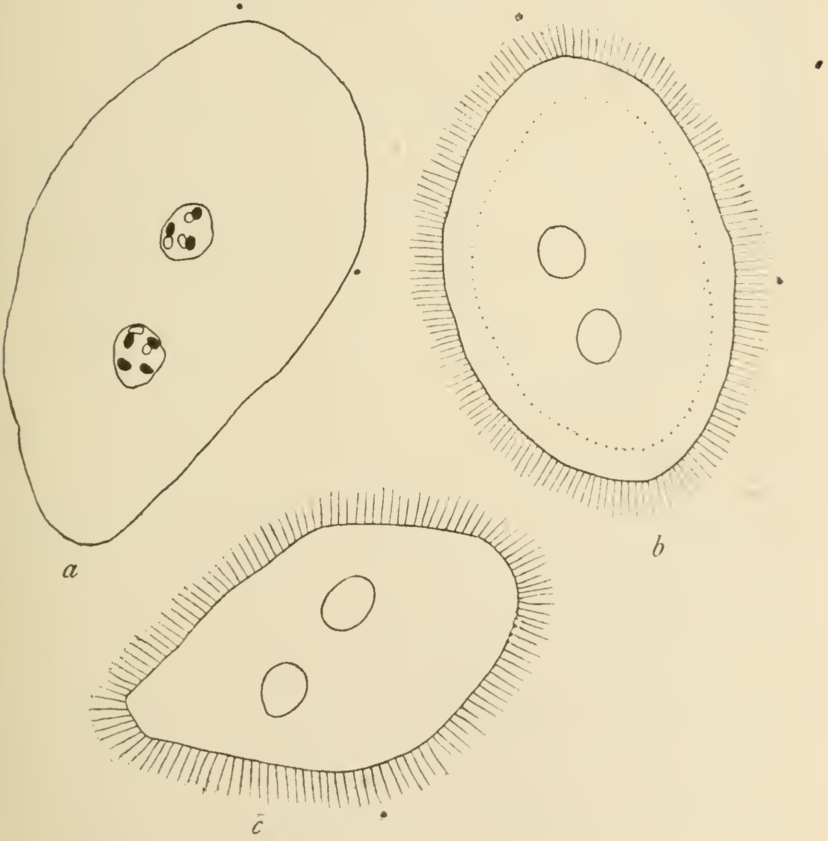

FIG. 56.—ZELLERIELLA COUCHII, × 460 DIAMETERS.

upon the basis of study of such scant material. With this species, in my material, are more numerous individuals of another, much smaller species, *Zelleriella atelopyxena*, to be described later.

ZELLERIELLA COUCHII, new species (fig. 56, p. 89).

Type.—United States National Museum Cat. No. 16463.

Host.—*Scaphiopus couchii* Baird, two scant infections in United States National Museum specimens both numbered 13629, from Helotes, Texas, November 30, 1883, G. W. Marnock, collector.

Measurements of a large individual.—Length of body 0.16 mm.; width of body 0.087 mm.; thickness of body 0.016 mm.; diameter of nucleus 0.0144 mm.; length of endospherule 0.0025 mm.; width of endospherule 0.002 mm.; cilia line interval, anterior 0.0016 mm.., middle 0.003 mm., posterior 0.0033 mm. Six, or possibly eight, chromosome masses are seen. The ectosarc is quite thick (fig. 56, *b*).

This is a smaller form than either *Z. hypopacheos* or *Z.* [*atelopodos*]. Its nuclei are relatively smaller and lie farther back in the body. Two shapes of the body may be noted, one (fig. 56, *a* and *c*) rather wedge-shaped and narrower than *Z. hypopacheos* or *Z.* [*atelopodos*], the other more rounded (fig. 56, *b*). In several nuclei six chromatin masses were fairly clearly seen. In another case, more obscure, there seemed to be eight. No nuclei were found in an anaphase of mitosis, the favorable phase for counting the chromosomes. It is unsafe to judge the number of chromosomes from any other stage of mitosis, though it is probable that six is the number of the massive chromosomes in this species.

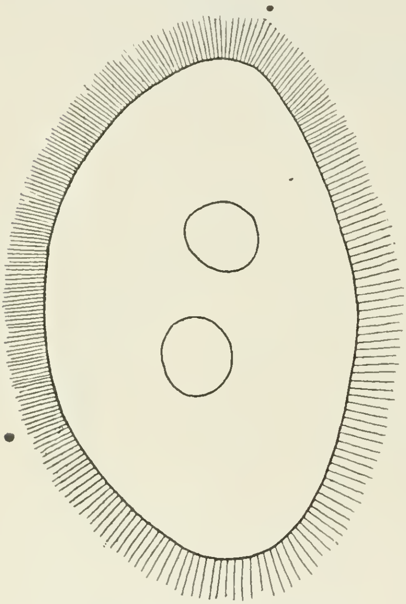

FIG. 57.—ZELLERIELLA SCAPHIOPODOS, × 460 DIAMETERS.

ZELLERIELLA SCAPHIOPODOS, new species (fig. 57, above).

Type.—United States National Museum Cat. No. 16464.

Host.—*Scaphiopus solitarius* Holbrook, from Raleigh, North Carolina, September, 1908; H. H. and C. S. Brimley, collectors.

Measurements.—Length of body 0.155 mm.; width of body 0.09 mm.; thickness of body 0.013 mm.; diameter of nucleus 0.0213 mm.; cilia line interval, anterior 0.0026 mm., posterior 0.00375 mm.

This species resembles *Z. couchii*, especially the broader individuals of the latter species, but has relatively much larger nuclei and must apparently be regarded as distinct.

Specimens of *Opalina oblanceolata* also are present in this same infection.

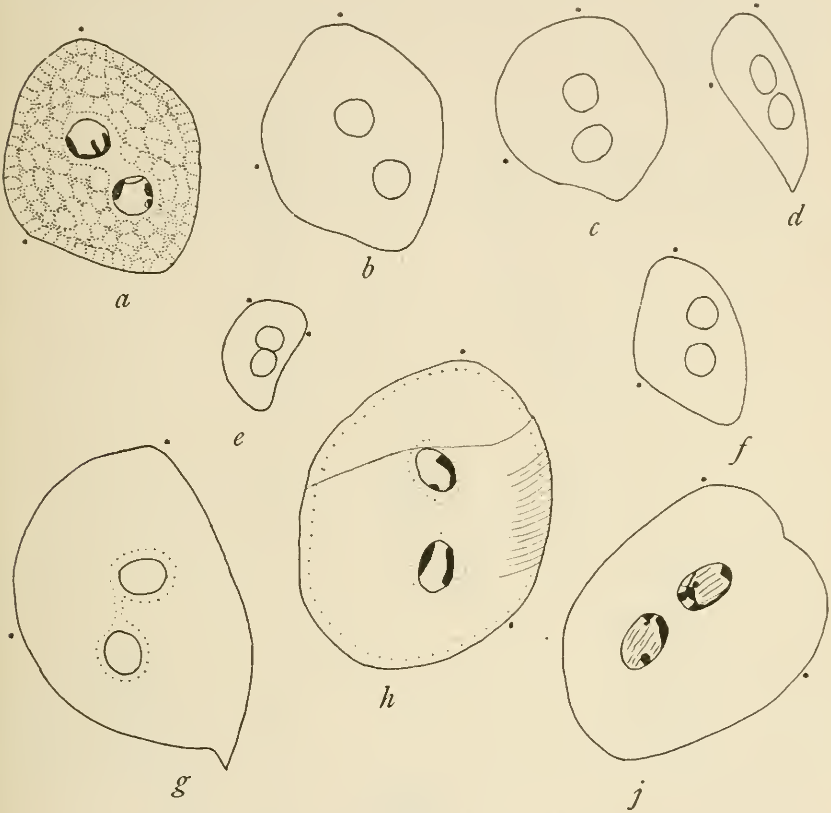

FIG. 58.—ZELLERIELLA ATELOPYXENA, × 460 DIAMETERS: *a, b, c, e, f*, DAUGHTER CELLS; *d, g, h*, INDIVIDUALS OF THE USUAL SORT; *j*, AN INDIVIDUAL APPARENTLY IN LONGITUDINAL FISSION, THOUGH ITS NUCLEI ARE NOT AS FAR ADVANCED AS USUAL AT THE TIME OF FISSION.

ZELLERIELLA ATELOPYXENA,[22] new species.

Type.—United States National Museum Cat. No. 16465.

Host.—*Atelopus varius* Stannius, two rather scant infections, from United States National Museum specimens No. 30643 (the type infection) and No. 30645, both from Pico Blanco, Costa Rica; W. M. Gabb, collector.

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[22] From the Greek ὁ ξένος, a male guest, or ἡ ξένη, a female guest.

Measurements: A, of a wedge-shaped individual; B, of a broader individual—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.097	0.091
Width of body	0.571	.083
Length of nucleus	.0135	.0135
Width of nucleus	.01	.0087

There are apparently eight massive chromosomes.

This small *Zelleriella* has two distinguishable forms, which, however, intergrade. In one form the individuals are broadly wedge-shaped (fig. 58, *d*, *g* and 59, *c*), usually with the posterior end distinctly pointed. The broad, rounded individuals are not usually pointed posteriorly. The nuclei are broadly ellipsoidal in the resting condition. In active mitosis the nuclei are more elongated, or spindle-shaped, or dumb-bell-shaped. Several daughter cells were found with both nuclei spherical. In several nuclei eight massive chromosomes were counted (fig. 59, *d*).

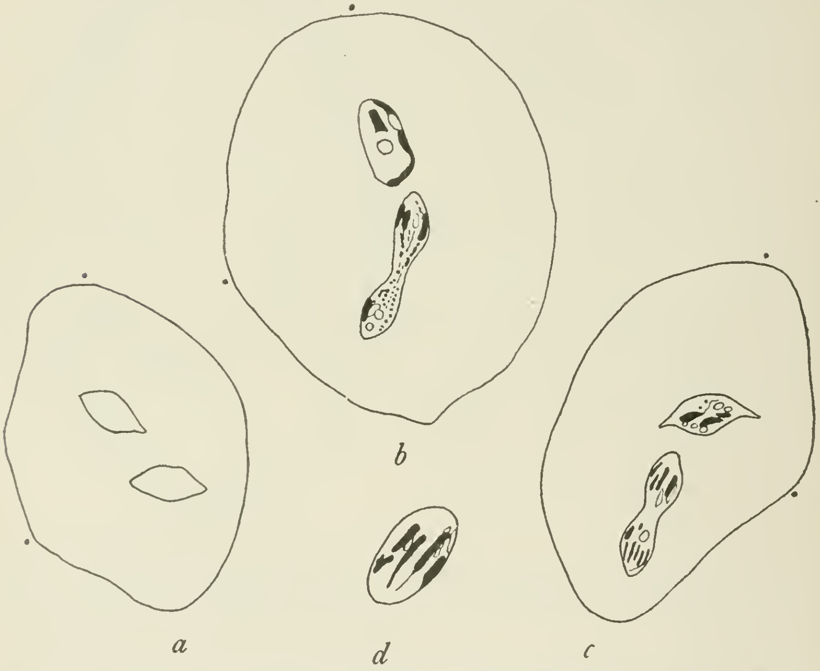


Fig. 59.—Zelleriella atelopyxena: *a to c*, individuals whose nuclei are in mitosis, × 460 diameters; *d*, a nucleus in mitosis showing 8 macrochromosomes, × 1,000 diameters.

ZELLERIELLA ATELOPYXENA [STELZNERI], new subspecies (?).

Type.—A specimen of this form has been deposited with the United States National Museum as No. 16466.

Host.—Atelopus stelzneri Weyenburgh, two infections, in United States National Museum specimens No. 28481 (the type infection, abundant) and No. 28521 (scant infection), both from Sapucay, Paraguay, W. T. Foster, collector.

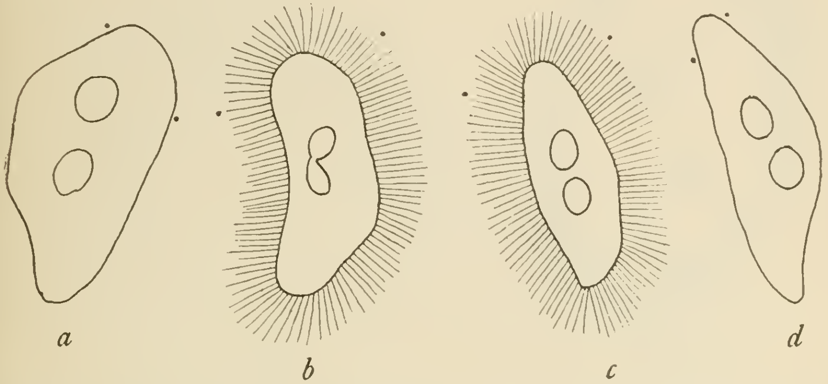


FIG. 60.—ZELLERIELLA ATELOPYXENA [STELZNER], × 460 DIAMETERS: *b* IS A DAUGHTER CELL.

Measurements: A, of a slender individual; B, of a broader individual—

Measurements.	A	B
	mm.	*mm.*
Length of body	0.087	0.08
Width of body	.028	.041
Length of nucleus	.013	.013
Width of nucleus	.0091	.012

This seems to be a slender variety of *Z. atelopyxena.* The posterior end of the body in most individuals is rounded, not pointed; in a few others it is distinctly pointed. The nuclei are rather similar in the species type and in its variety. I am naming the variety only tentatively, pending further study of more abundant and better material.

ZELLERIELLA [ENGYSTOMOPSIS], new species (?) (figs. 61 and 62).

Type.—A specimen of this form has been deposited with the United States National Museum as Cat. No. 16467.

Hosts.—Engystomops stentor (Espada), three infections, in United States National Museum specimens No. 51959 (the type infection, abundant), Nos. 51957 and 51958, all from Taboga Island, Panama, June 12, 1914, J. Zetek, collector; also *Engystomops pustulosus*

(Cope), three abundant infections, two from Tehuantepec, Mexico, F. Sumichrast, collector, and the other labeled merely "Mexico," F. Sumichrast, collector. The individuals of this third infection are poorly preserved and have not been studied in detail.

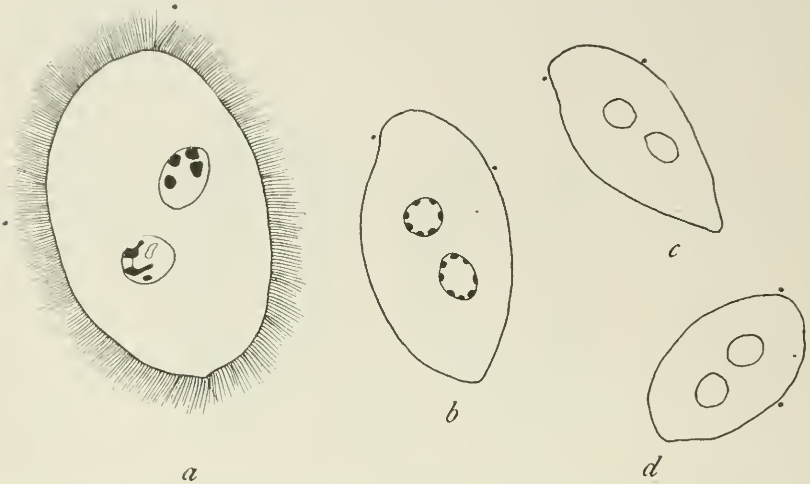


FIG. 61.—ZELLERIELLA [ENGYSTOMOPSIS], FROM ENGYSTOMOPS STENTOR, × 460 DIAMETERS.

Measurements: A, of a large individual of the broader form; B, of a full-sized individual of the more wedge-shaped form; C, of an individual in division—

Measurements.	*A*	*B*	*C*
	mm.	*mm.*	*mm.*
Length of body	0.0978	0.07	0.087
Width of body	.062	.033	.046
Thickness of body	.019	.0165	.019
Length of nucleus	.019	.013	.011
Width of nucleus	.014	.0097	.009
Diameter of endospherule	.0015		
Cilia line interval:			
Anterior	.00182		.002
Posterior			.004

Many individuals of this species, even some of the broadest, are pointed posteriorly (figs. 61*a* and 62). In some cases the point is abrupt and small. The number of the massive chromosomes can not confidently be stated from the preparations available for study. Further study of a larger series of infections may show this form to be very close to, or possibly identical with, *Z. couchii*. I am therefore bracketing the specific name.

ZELLERIELLA DENDROBATIDIS, new species.

Type.—United States National Museum Cat. No. 16468.

Hosts.—*Dendrobates tinctorius* (Schneider), three infections, in United States National Museum specimens No. 32535, 39 mm. long (the type infection, abundant), from Costa Rica, Gabb, collector; No. 19771 and No. 19772, both from Greytown, Nicaragua, April 10. 1892, C. W. Richmond, collector; and *Dendrobates typographus* Keferstein, six infections in United States National Museum specimens Nos. 30585, 32322, 19588, 19590, 19652, and 19653, all from Nicaragua or Costa Rica.

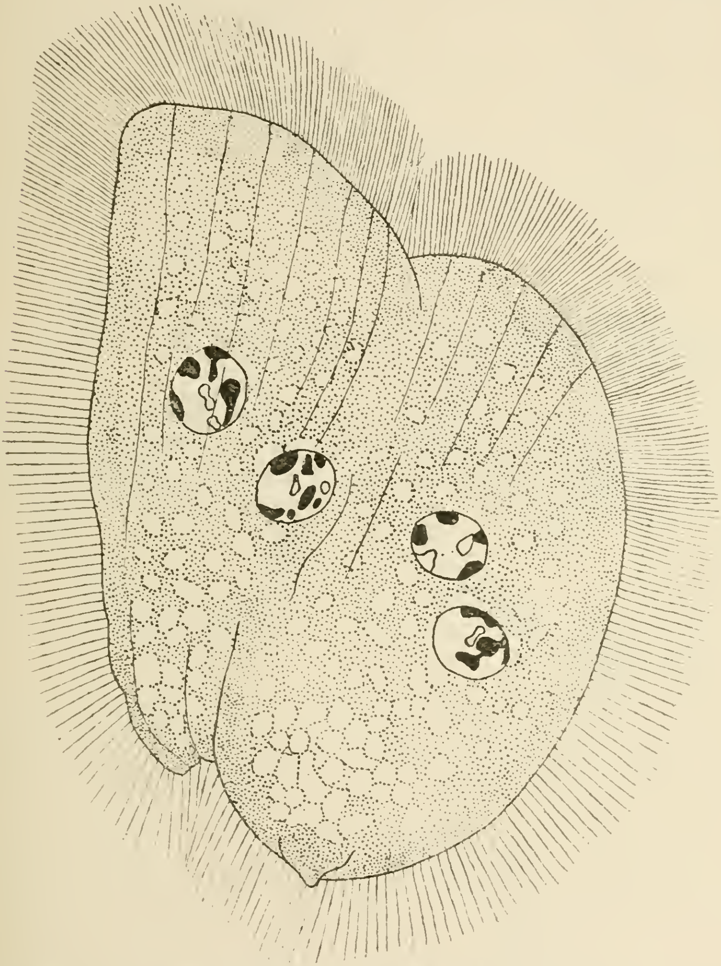


Fig. 62.—Zelleriella [Engystomopsis], from Engystomops stentor, in longitudinal fission, × 1,000 diameters.

Measurements of an average individual.—Length of body 0.077 mm.; width of body 0.05 mm.; thickness of body 0.014 mm.; diameter of nucleus 0.01 mm.; diameter of endospherule 0.0015 mm.; cilia line interval, anterior 0.002 mm., posterior 0.003 mm.

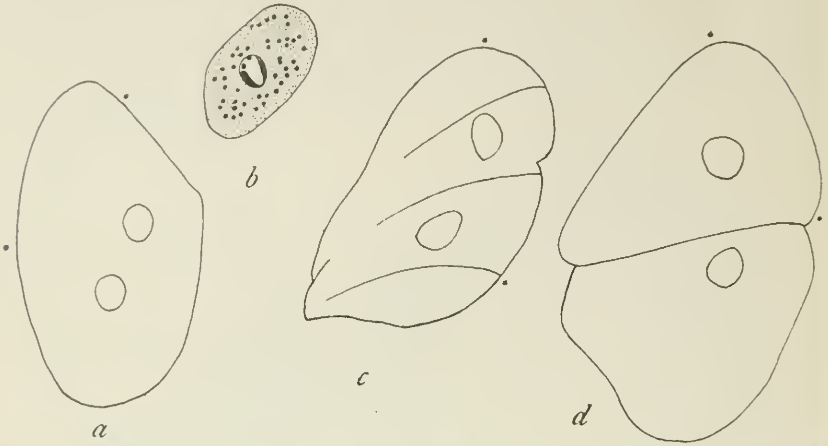


Fig. 63.—Zelleriella dendrobatidis, from Dendrobates typographus, × 460 diameters: *c*, in longitudinal fission; *d*, in transverse fission. Three lines of insertion of cilia are indicated in *c*.

This form is rather similar to *Z.* [*engystomopsis*], but probably is distinct. In my material the nuclei of individuals in fission are each oval or spindle-shaped, while in dividing individuals of *Z.* [*engystomopsis*] the nuclei are usually in a much more advanced condition, each forming daughter cell already showing two separated, spheroidal, telophase nuclei. In general among the binucleated Opalinids comparison of the mitotic phases of the nuclei in individuals of corresponding general condition is one of the safest tests of specific distinctness.

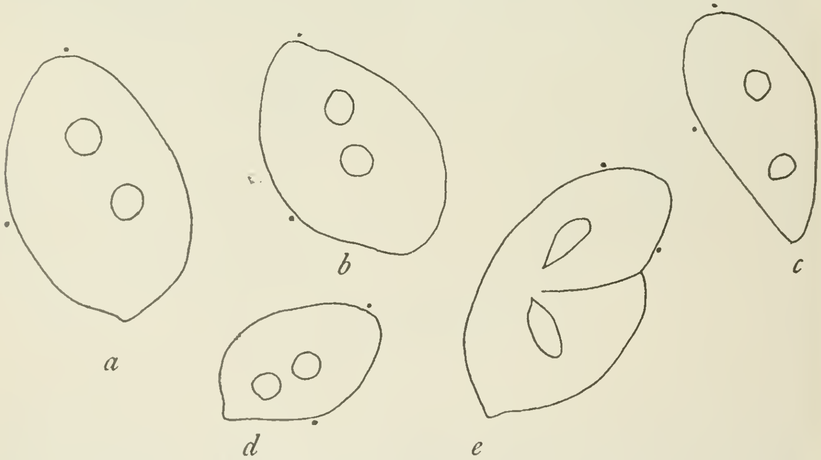


Fig. 64.—Zelleriella dendrobatidis, from Dendrobates tinctorius, × 460 diameters: *e*, is in transverse fission.

ZELLERIELLA LEPTODACTYLI, new species.

Type.—United States National Museum Cat. No. 16469.

Hosts.—Four species of *Leptodactylus: L. albilabris* (Günther), six abundant infections from Porto Rico, Vicuez Island, and Tehuantepec, Mexico; *L. caliginosus* Girard, one abundant infection from Tehuantepec,, Mexico; *L. gracilis* (Duméril and Bibron), two abundant infections from Tehuantepec, Mexico; and *L. microtis* (Cope), four good infections from Lake Amatitlan, Guatemala. These infections were all in specimens from the United States National Museum and are listed in section 8 of this paper. The type infection is from *L. albilabris* No. 10029, four specimens, one uninfected, one scantily, and two abundantly infected. They are from Tehuantepec, Mexico; F. Sumchrast, collector. Specimens of this Opalinid from each of the other hosts are deposited in the United States National Museum as follows: Cat. No. 16633 (from *L. caliginosus*); Cat. No. 16634 (from *L. gracilis*); Cat. No. 16635 (from *L. microtis*).

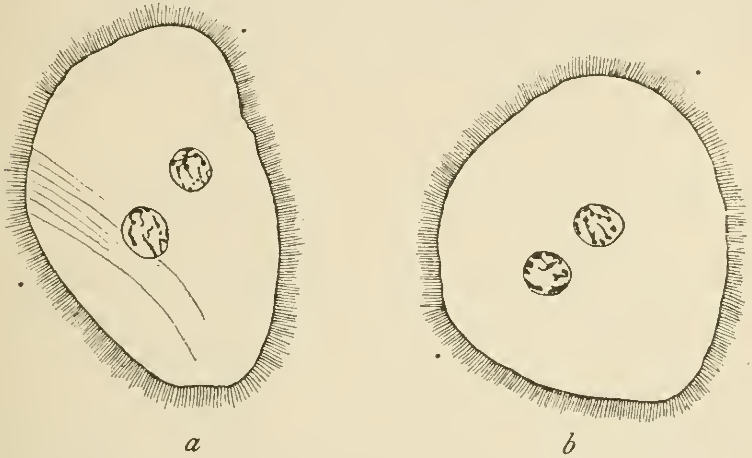


FIG. 65.—ZELLERIELLA LEPTODACTYLI, FROM LEPTODACTYLUS ALBILABRIS, × 460 DIAMETERS.

Measurements of an average individual from Leptodactylus albilabris.—Length of body 0.105 mm.; width of body 0.067 mm.; thickness of body 0.017 mm.; diameter of nucleus 0.013 mm.; cilia line interval, anterior 0.0011 mm., posterior 0.0028 mm.

In this species the posterior end of the body is not pointed in any individuals. In one well-preserved infection from *Leptodactylus albilabris* the nuclei are seen to be resting in a condition that is a transition from the reticulate to the skein phase of mitosis.

The single infection from *L. caliginosus* does not show the nuclei. Either the preservation is poor, or the nuclei are degenerate (possibly as Neresheimer has described for two multinucleate species?). The cilia line intervals in an average individual from this host are different from those of an average individual from *L. albilabris*, judging from my material from the two hosts, the intervals for *L. caliginosus* being; cilia line interval, anterior 0.0018 mm., posterior 0.00375 mm. A difference so marked is probably significant, but it hardly seems best to name a variety upon the basis of this variable character, especially as the absence of demonstrable nuclei makes the real nature of these forms very doubtful.

The material from the two infections of *Leptodactylus gracilis* is not very well preserved. I have noted no distinctions from the material in *L. albilabris*, except that the individuals from the latter host are for the most part noticeably broader.

The material from *Leptodactylus microtis* also is poorly preserved, the nuclei being shrunken, with crinkled membranes, causing them to appear smaller. The Opalinids in both infections are considerably smaller than in *L. albilabris*. It is not improbable that study of abundant living material would show specific difference.

ZELLERIELLA BRASILIENSIS (Pinto).

Opalina brasiliensis PINTO (1918).

A specimen of this species has been deposited with the United States National Museum as Cat. No. 16470.

Host.—*Leptodactylus ocellatus* (Linnaeus), Pinto (1918) described this species from Rio de Janeiro, Brazil. My material contains one good infection from United States National Museum specimen No. 22749, 76½ mm. long, from La Plata, Argentina.

Measurements of a medium-sized individual from my material: Length of body 0.13 mm.; width of body 0.082 mm.; length of resting nuclei 0.0152 mm.; width of resting nuclei 0.0109 mm.; cilia line interval, anterior 0.00195 mm., middle 0.00237 mm., posterior 0.00275 mm. Four massive chromosomes.

The majority of the individuals in my infection are longer and relatively narrower than *Zelleriella leptodactyli.* Some, however, show the broad form, but none so very broad as the broadest forms of the latter species. The resting nuclei are ovoid. Large individuals with spindle-shaped or dumb-bell-shaped anaphase nuclei are found, also daughter cells with dumb-bell-shaped anaphase nuclei. These conditions indicate that fission usually is consummated while the nuclei are in the dumb-bell anaphase condition, though Pinto describes a still undivided individual with four independent daughter nuclei. One wonders if this individual may have been kept for a time outside the host and so had its completion of the division of the body hindered (see Metcalf, 1909). In a number of the anaphase nuclei, both spindle-shaped and dumb-bell-shaped, four massive chromosomes are plainly seen. I had studied and named this species "*Zelleriella argentinae*" before I saw Pinto's paper, but as his animals and mine seem of the same sort, his name is, of course, accepted.

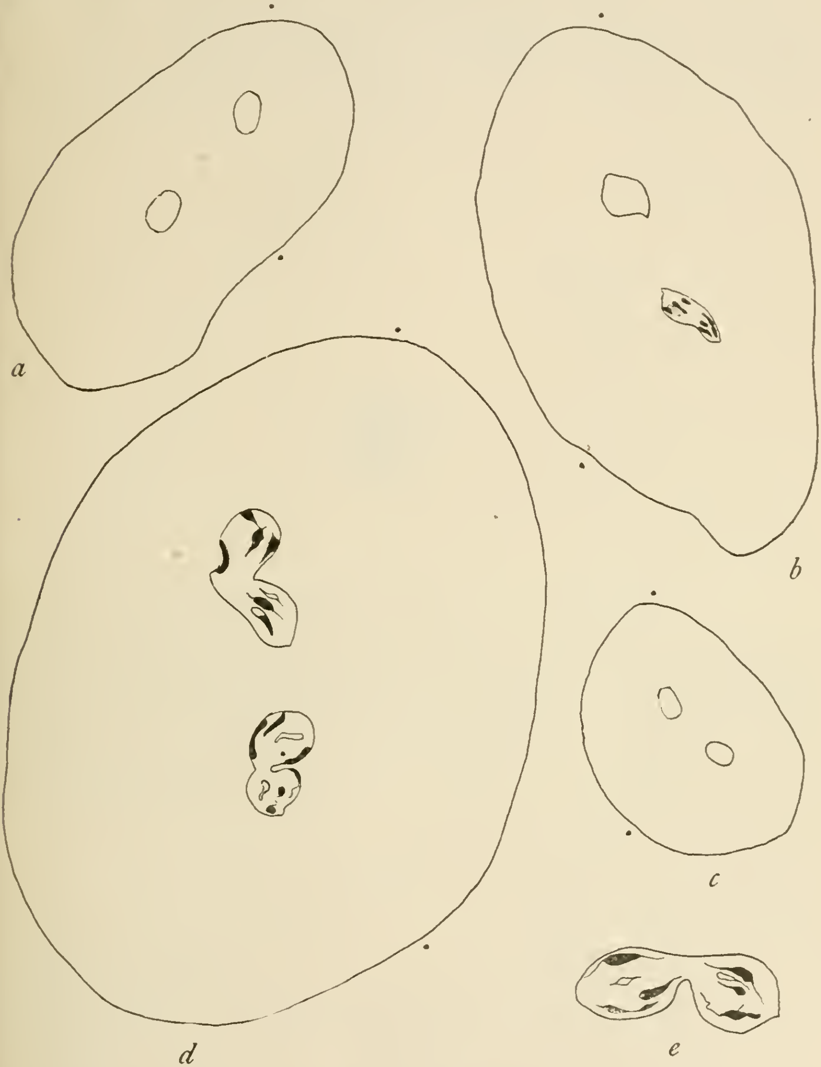


Fig. 66.—Zelleriella brasiliensis: *a* and *c*, individuals of the usual sort; *b*, a cell with nuclei in an anaphase of mitosis preparatory to fission; *d*, a cell with dumb-bell nuclei; *e*, a dumb-bell nucleus from another individual; *a*, *b*, and *c*, magnified 460 diameters; *e*, magnified 1,000 diameters.

ZELLERIELLA MAGNA, new species.

Type.—United States National Museum Cat. No. 16471.

Host.—Leptodactylus typhonius (Daudín), one abundant infection, in United States National Museum specimen No. 36370, 44 mm. long;

a male with huge fat bodies, from Chicara, Venezuela, "Brooklyn Institute."

Measurements of an ordinary individual.—Length of body 0.27 mm.; width of body 0.13 mm.; length of nucleus 0.0325 mm.; width of nucleus 0.0228 mm.; diameter of endospherule 0.0023 mm.; cilia line interval, anterior 0.00216 mm., posterior 0.00425 mm.

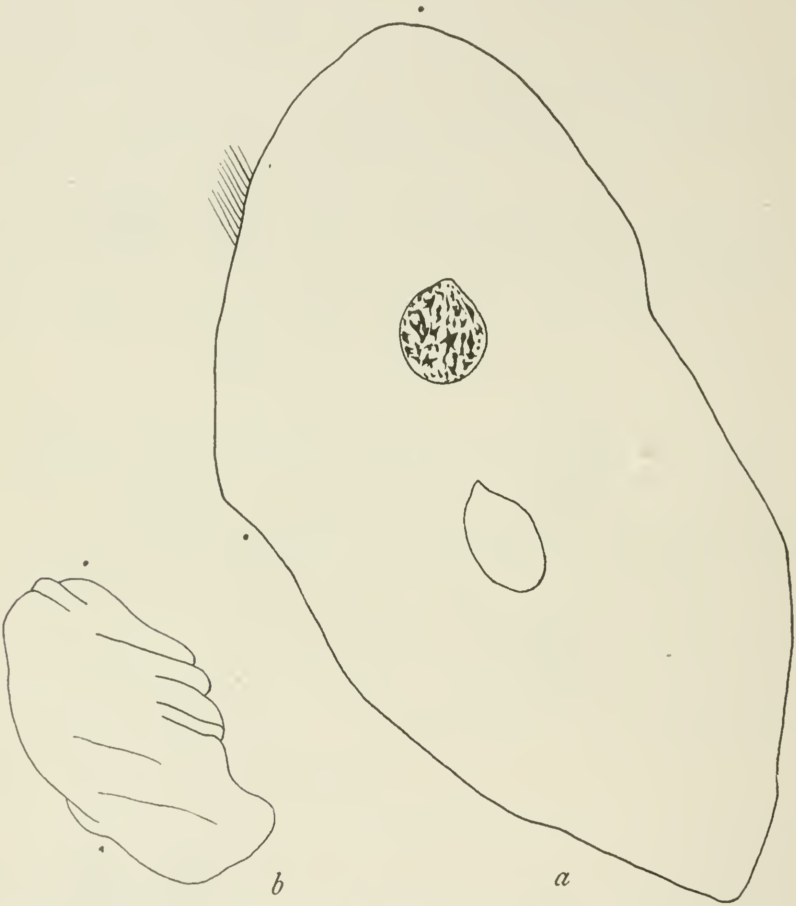


FIG. 67.—ZELLERIELLA MAGNA: *a* IS MAGNIFIED 460 DIAMETERS; *b* IS MAGNIFIED 249 DIAMETERS.

This very thin species is the largest known *Zelleriella* except *Z. opisthocarya* (to be described). It is seen in my material in two conditions, wedge-shaped individuals, as in figure 67, *a*, and ridged, crenate, slightly twisted individuals (fig. 67, *b*), such as are so often found in life in many species of flattened Opalinids and in some cylindrical species as well. Probably prompt action of the killing fluid has in this material preserved the crinkled form of the animals. The nuclei in the many individuals observed are sometimes ellipsoid, but much more usually somewhat pear-shaped. These nuclei are resting in a highly reticulate late metaphase. The endospherules are large in this species.

ZELLERIELLA [BOULENGERI], new species (?).

A specimen of this form has been deposited with the United States National Museum as Cat. No. 16472.

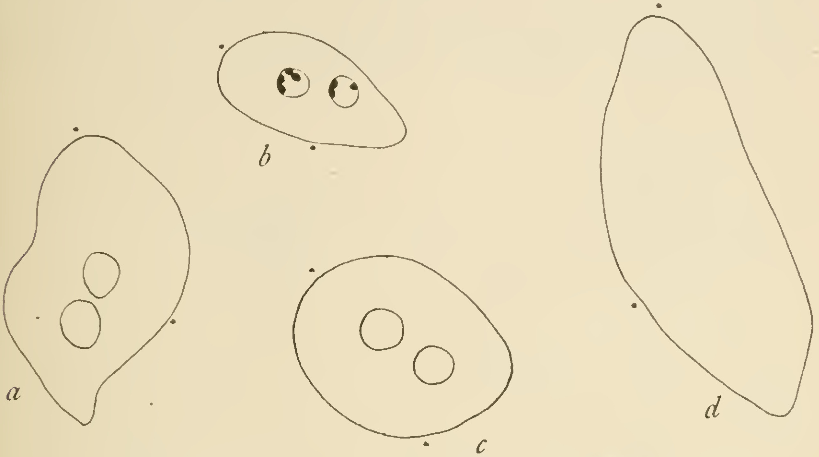


FIG. 68.—ZELLERIELLA [BOULENGERI], × 460 DIAMETERS.

Host.—Prostherapis boulengeri Barbour, one infection in United States National Museum specimen No. 52406, 22 mm. long, from Gorgona Island, Colombia, South America.

Measurements: A, of a small individual; B, of one of medium size; C, of the largest one observed—

Measurements.	*A*	*B*	*C*
	mm.	*mm.*	*mm.*
Length of body	0.057	0.063	0.111
Width of body	.028	.047	.047
Thickness of body	.0145	.0155	
Length of nucleus	.0087	.0087	
Width of nucleus	.0087	.0054	
Cilia line interval:			
Anterior		.00187	
Posterior		.003	

This *Zelleriella* seems very similar to *Z. atelopyxena* [*stelzneri*], but its nuclei are spherical in almost all individuals, instead of ellipsoidal. They seem to be resting in a late metaphase, entering upon the reticulate condition, but the preservation is not good enough to allow very clear vision of the cytological phenomena.

ZELLERIELLA PALUDICOLAE, new species.

Type.—United States National Museum Cat. No. 16473.

Hosts.—*Paludicola bibronii* (Tschudi), eight infections, three abundant, from Tolcahuano, Chile, Barbour, collector; of these, United States National Museum specimen No. 38920 furnished the type infection. *Paludicola brachyops* (Cope), three infections, one abundant, from Chiara, Venezuela, June 6, 1901, G. K. Cherrie, collector; another, abundant, from Margarita Island, Venezuela, July 3, 1895, Robinson, collector; and one, scant, from "South America," Brooklyn Institute. A specimen of this Opalinid from *P. brachyops* has been deposited with the United States National Museum as Cat. No. 16474.

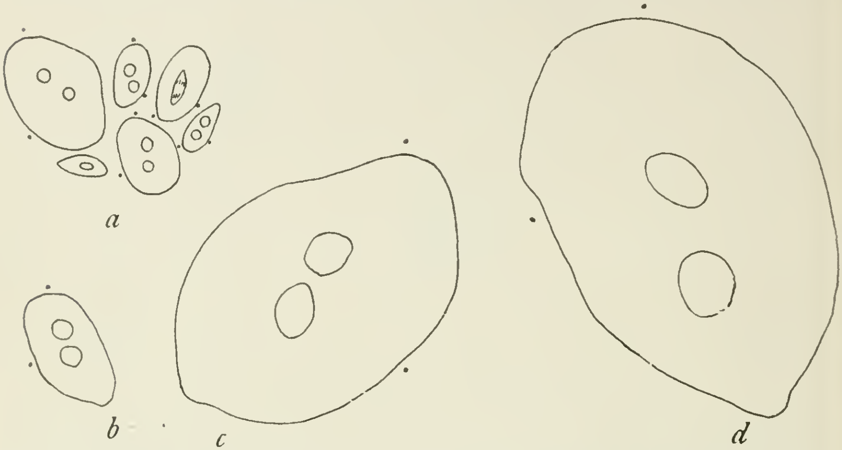


FIG. 69.—ZELLERIELLA PALUDICOLAE, FROM PALUDICOLA BIBRONII: *a*, A GROUP OF CELLS MAGNIFIED 117 DIAMETERS; *b, c, d,* CELLS MAGNIFIED 430 DIAMETERS.

Measurements: A, of a large individual with resting nuclei; B, of a large daughter cell with nuclei in an equatorial plate phase of mitosis—

Measurements.	A	B
	mm.	*mm.*
Length of body	0.147	0.01435
Width of body	.104	.08
Length of nucleus	.0175	.031
Width of nucleus	.014	.012
Length of endospherule	.0024	....
Width of endospherule	.001	....
Cilia line interval:		
Anterior	.0023	....
Posterior	.00365	....

Ten macrochromosomes.

This is a broad *Zelleriella*, with the posterior end of the body not pointed, but a few of the smallest daughter cells, apparently the result of rapid division, are more slender and wedge-shaped, with the posterior end tapering to a rounded, never a sharp, point. The resting nuclei are nearly spherical, being but little elongated in one diameter. Fission occurs while the two nuclei are but little more advanced than this in mitosis. The nuclei of the daughter cells thus formed at once enter upon mitosis, so that we find some of them with slightly elongated nuclei, some with more ellipsoidal nuclei, others with spindle-shaped nuclei in the equatorial plate stage

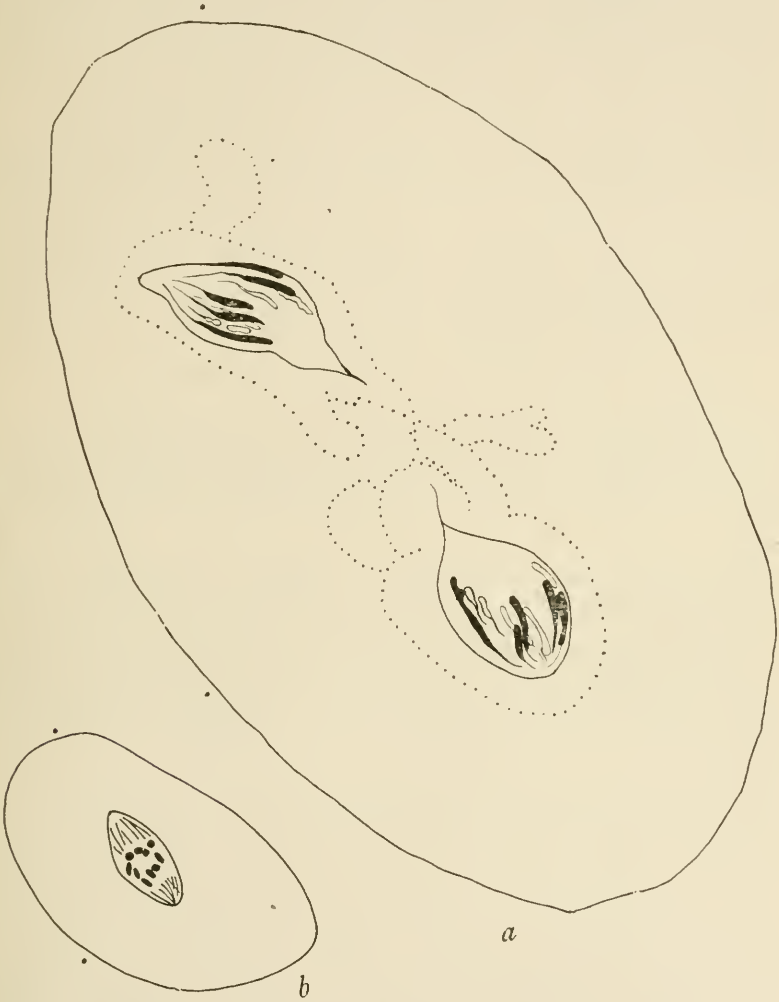


FIG. 70.—ZELLERIELLA PALUDICOLAE, FROM PALUDICOLA BIBRONII; *a*, MAGNIFIED 1,000 DIAMETERS; *b*, A DAUGHTER CELL MAGNIFIED 460 DIAMETERS.

(unusually clear in this species, fig. 70, *b*), and others with spindle-shaped anaphase nuclei; dumb-bell nuclei are less common. The macrochromosomes have been counted in a good many of the equatorial plate and anaphase nuclei and their number found to be 10. (Fig. 70, *a* and *b*.)

All my material of this species from *Paludicola bibronii* was collected at one time from one locality and all the infections show active division, with many daughter cells whose nuclei are in active division, restoring the animals to their binucleated resting condition. The infections from *P. brachyops* show the numerous daughter cells with their single nuclei more or less ellipsoidal and in some prophase of mitosis. It is possible a varietal distinction might be made between the Zelleriellas in the two hosts; but if so, a larger series of infections from both hosts should be studied to see if the conditions are constant. The slight distinction observed might better, for the present at least, be treated as a racial divergence.

ZELLERIELLA PATAGONIENSIS, new species.

Type.—United States National Museum Cat. No. 16475.

Host.—*Paludicola bufonina* (Bell), six good infections from the Straits of Magellan, Patagonia, 1898; Hatcher, collector. The type infection is from United States National Museum specimen No. 36883.

Measurements of a medium-sized individual.—Length of body, 0.152 mm.; width of body, 0.091 mm.; diameter of nucleus, 0.0156 mm.; diameter of endospherule, 0.0016 mm.; cilia line interval, anterior 0.0021 mm., posterior 0.000375 mm. Macrochromosomes apparently eight. Larger individuals up to 0.170 mm. are found, these having their nuclei slightly elongated, apparently preparatory to mitosis.

These Zelleriellas are found in two forms—one wedge-shaped, with usually a short, abrupt, but definite posterior point, not usually sharp; the other very broad and rounded posteriorly. Intergrades between these two extremes are seen. All are very thin. The resting nuclei are nearly spherical. In two uninucleated individuals, dumb-bell-shaped nuclei were studied—one in side view, the other in end view—each showing eight unusually spheroidal macrochromosomes, short, broad, and thick, instead of ribbon-shaped, as is usual in the Opalinids. This form of the chromosomes, each compacted into a sphere, may be the result of abnormal conditions. Possibly the hosts were dead for some time before they were placed in the preserving fluid. The study of the macrochromosomes in living individuals of other Opalinids has shown them very sensitive to abnormal conditions, several peculiar phenomena appearing. Among these, rounding up of the macrochromosomes is often seen.

Zelleriella patagoniensis is much like *Z. paludicolae*, but in my infections is larger and its body form is different in some individuals. Figure 71, *c*, shows an individual of a form very frequent in *Z. patagoniensis*, but not observed in *Z. paludicolae*. The comparison between these two species is something like that between *Protoopalina caudata* and *P. intestinalis*. The pointed tip, or posterior process, so often seen in *P. caudata*, though not in all individuals, is one of the most characteristic things in that species and is not observed in any individuals of *P. intestinalis*. Similarly, many, but not all, individuals of *Zelleriella patagoniensis* show the definite posterior process, short and flat, to be sure, in this flat species, while in *Z. paludicolae* no such posterior process is found at all well developed.

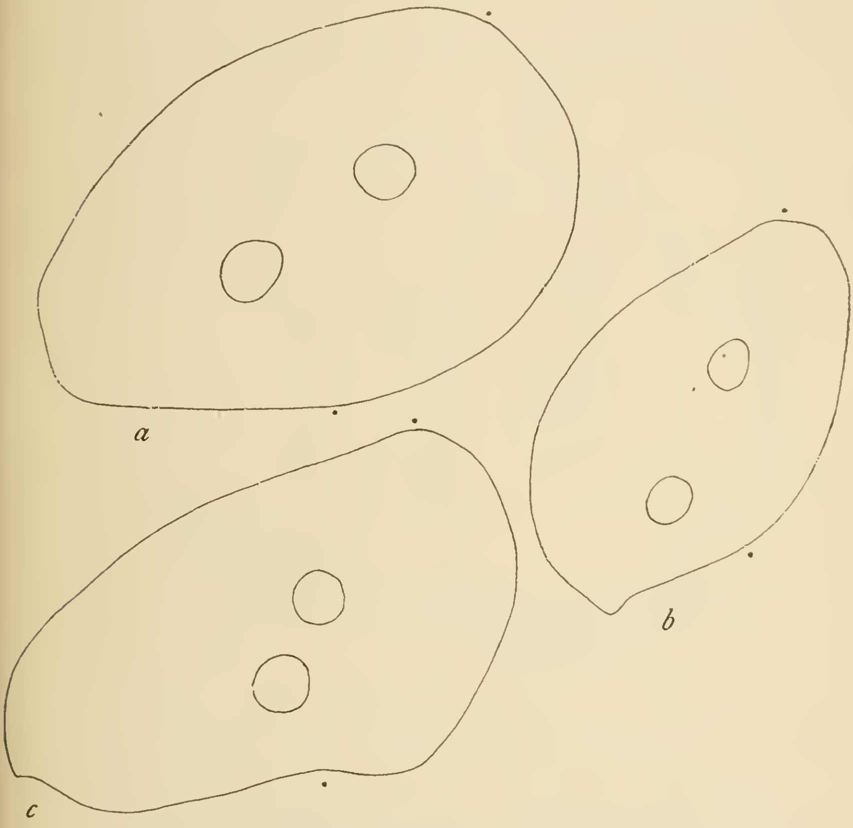


FIG. 71.—ZELLERIELLA PATAGONIENSIS, × 460 DIAMETERS.

ZELLERIELLA RANAXENA, new species.

Type.—United States National Museum Cat. No. 16476.

Host.—*Rana draytonii* Baird and Girard, from California, April, 1913. Purchased frogs, collector and exact locality unknown.

Measurements of an ordinary individual.—Length of body, 0.1674 mm. (of a large individual 0.187 mm.); width of body, 0.091 mm.; diameter of nucleus, 0.0174 mm.; cilia line interval, anterior 0.003 mm., posterior 0.0045 mm.

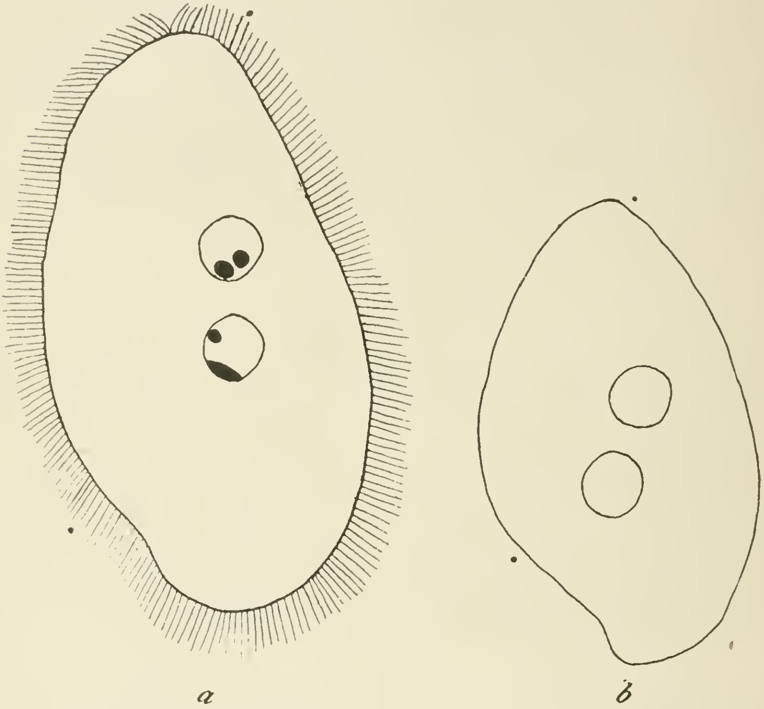


FIG. 72.—ZELLERIELLA RANAXENA, × 460 DIAMETERS.

The characteristic form of this rather large and very thin *Zelleriella* is shown in figure 72, *b*. Its shape is rather similar to that of *Z. patagoniensis*, except that the posterior end of the body, when pointed at all, is always rounded, never sharp. It seems clearly a distinct form. No nuclei in mitosis were observed.

ZELLERIELLA DARWINII, new species (figs. 73 and 74).

Type.—United States National Museum Cat. No. 16477.

Host.—*Rhinoderma darwinii* Dumeril and Bibron, one infection in United States National Museum specimen No. 38931, 32 mm. long, from Concepcion, Chile; T. Barbour, collector.

Measurements of an ordinary binucleated individual.—Length of body 0.2 mm.; width of body 0.115 mm.; thickness of body 0.021 mm.; length of nucleus 0.024 mm.; width of nucleus 0.017 mm.; length of endospherule 0.0024 mm.; width of endospherule 0.0016 mm.; cilia line interval, anterior 0.00161 mm., posterior 0.0035 mm.

This is a large *Zelleriella* which, except during and after division, has about the form shown in figure 73. The mitotic condition of the broadly ellipsoidal nuclei seems to be a granular prophase, but I suspect that all the nuclei are abnormal, probably because of abnormal environmental conditions before their preservation. All the elongated nuclei seen in dividing individuals or daughter cells, as well as the more spheroidal nuclei of the ordinary individuals, show an irregularly granular condition of the chromatin which is different from the normal conditions of the nuclei of other species in the corresponding phases. Fission evidently is consummated while the nuclei are elongated ellipsoidal, or almost spindle-shaped.

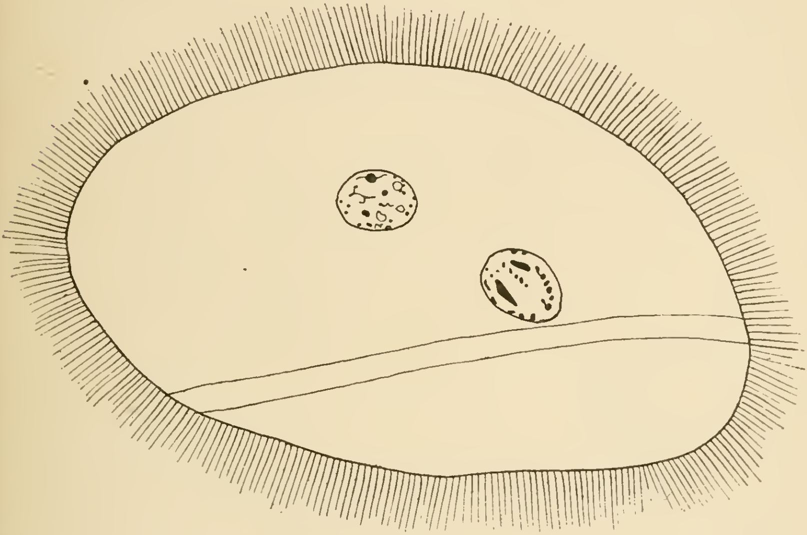


FIG. 73.—ZELLERIELLA DARWINII, A CELL OF THE USUAL SORT, × 460 DIAMETERS. TWO LINES OF INSERTION OF CILIA ARE SHOWN.

ZELLERIELLA CUSCONIS, new species (fig. 75, p. 109).

Type.—United States National Museum Cat. No. 16478.

Host.—*Eleutherodactylus footei* Stejneger, one infection, from United States National Museum specimen No. 49563, 19 mm. long, from Cuzco, Peru, 11,500 feet altitude, July 9; Yale Peruvian Expedition, collectors.

Measurements: A, of a large individual; B, of a medium-sized individual—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.13	0.09
Width of body	.087	.044
Length of nucleus	.03	.0185
Width of nucleus	.0152	.012

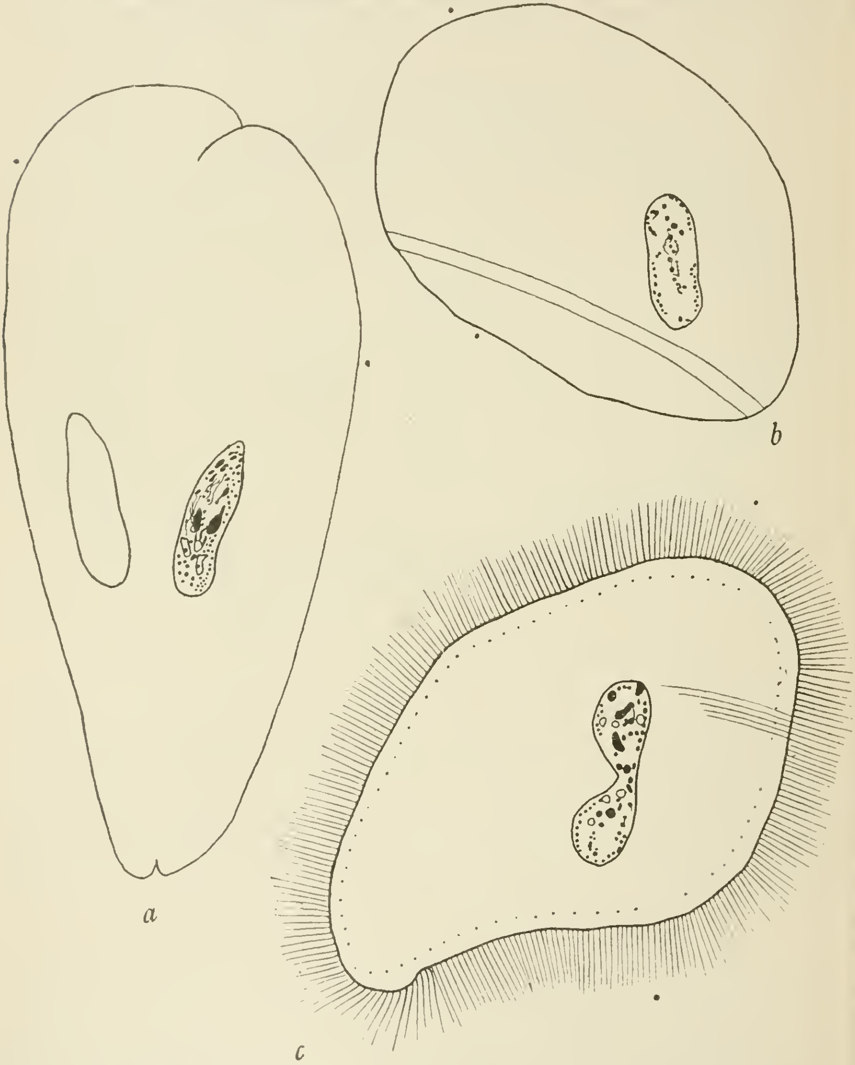


FIG. 74.—ZELLERIELLA DARWINII, × 460 DIAMETERS: *a* IS IN LONGITUDINAL FISSION; *b* AND *c* ARE DAUGHTER CELLS; *b* SHOWS TWO OF THE PRIMARY LINES OF INSERTION OF CILIA; IN *c* ALL THE LINES OF INSERTION OF CILIA ARE DRAWN FOR A SMALL AREA, SHOWING HOW THE LINES BECOME CROWDED ANTERIORLY BY THE INTERPOLATION OF ACCESSORY LINES.

This material is poorly preserved and the intranuclear phenomena are not well seen. The form of the body is sufficiently well indicated in the figures. In most individuals, even in some of the broadest ones, there is a posterior point with rounded tip. In the ordinary individuals we find two nuclei, each more or less elongated ellipsoidal. In the daughter cells we see one similar nucleus, or a single more elongated spindle-shaped nucleus, or two nearly spherical daughter nuclei not connected by a thread. It seems then that fission is consummated while the nuclei are ellipsoidal, nearly twice as long as broad.

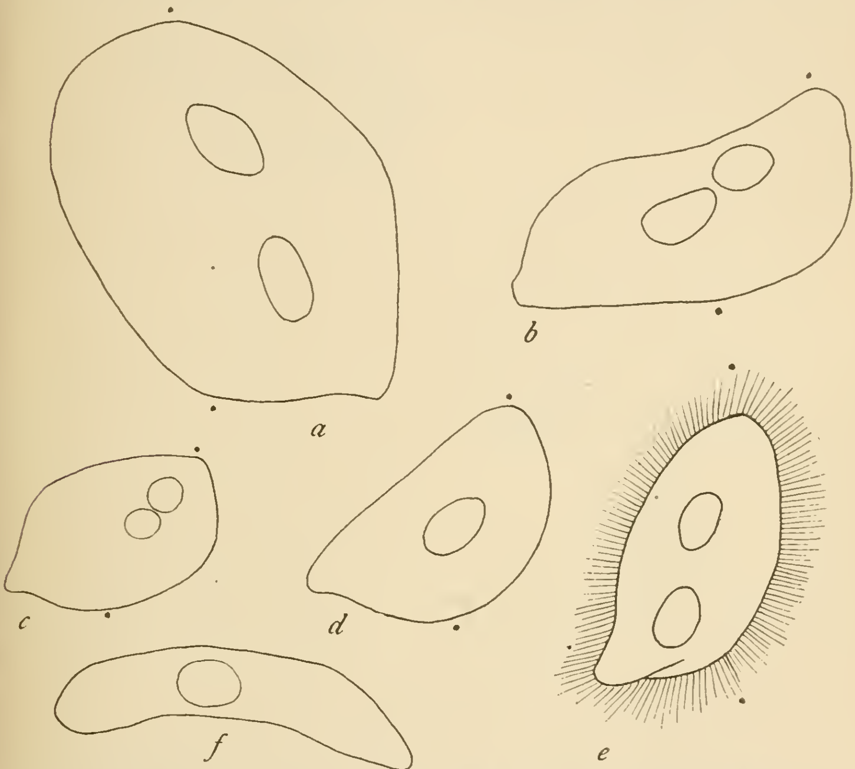


FIG. 75.—ZELLERIELLA CUSCONIS, × 460 DIAMETERS: *a*, AN INDIVIDUAL ALMOST READY FOR FISSION; *b* AND *e*, CELLS OF THE USUAL SORT; *c* AND *d*, DAUGHTER CELLS; *f*, AN OPTICAL, LONGITUDINAL SECTION OF THE BODY THROUGH ONE NUCLEUS, SHOWING THE THICKNESS OF THE BODY.

In body form *Zelleriella cusconis* resembles *Z. dendrobatidis* and *Z.* [*boulengeri*], but its resting nuclei in ordinary individuals are ellipsoidal and not spherical as in the latter two species.

ZELLERIELLA BINGHAMI, new species.

Type.—United States National Museum Cat. No. 16479.

Host.—*Eleutherodactylus binghami* Stejneger, the type infection, abundant, from United States National Museum specimen No. 49558, 32 mm. long, and one other, from Urubamba, Peru, 9,500 feet altitude, July 15, Yale Peruvian Expedition, collectors; and another scant infection from United States National Museum specimen No. 48560, 23 mm. long, from Cuzco, Peru, 11,500 feet altitude, July 9, Yale Peruvian Expedition, collectors.

Measurements of an ordinary individual.—Length of body 0.093 mm.; width of body 0.0465 mm.; diameter of nucleus 0.014 mm.; cilia line interval, anterior 0.002 mm.; posterior 0.00312 mm.

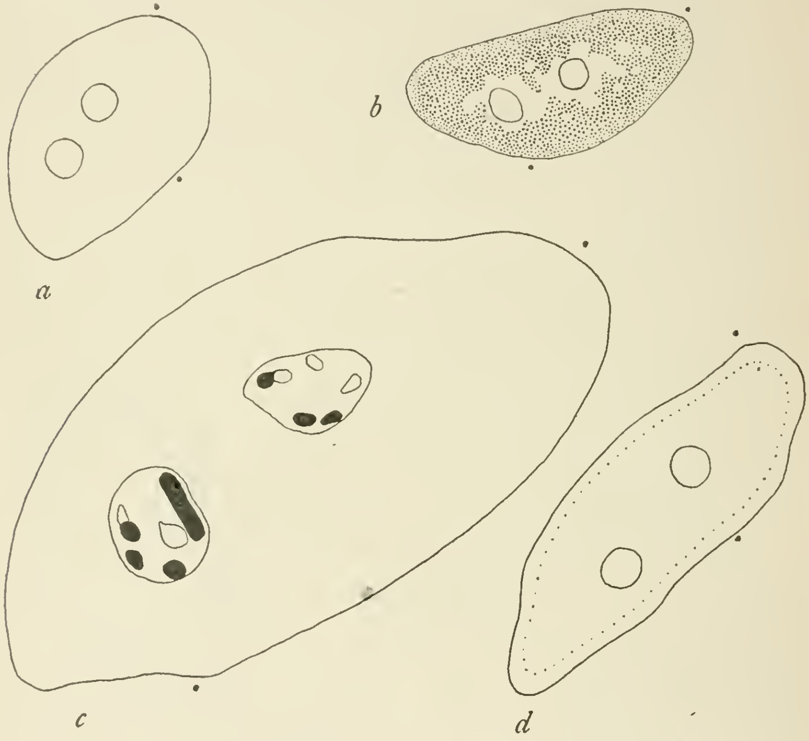


FIG. 76.—ZELLERIELLA BINGHAMI: *a*, *b*, AND *d*, CELLS OF THE ORDINARY SORT, × 460 DIAMETERS; *c*, A CELL ENTERING UPON MITOSIS, AS INDICATED BY THE SLIGHT ELONGATION OF THE NUCLEI, × 1,000 DIAMETERS.

This small *Zelleriella* is in general of the form shown in the figures, though some rather broader individuals are seen. All the individuals observed in my material have two spheroidal nuclei, except a few in which the nuclei are just a little elongated (fig. 76, *c*). In two of the latter sort, six chromatin masses were observed in each nucleus, which suggests that there are probably six macrochromosomes, but one can not be confident of this without studying the mitotic cycle, especially the anaphase, and my material does not allow this. I do not understand the huge chromosome in the posterior nucleus shown in figure 76, *c*.

This seems a species quite distinct from those described, its body form and nucleus form being characteristic.

ZELLERIELLA [TRINITATIS], new species (?).

A specimen of this form has been deposited with the United States National Museum as Cat. No. 16494.

Host.—*Phyllobates trinitatis* Garman, two infections, the type infection from United States National Museum specimen No. 27792, 23 mm. long, from La Guaira, Venezuela, July 10, 1900, Lyon and Robinson, collectors, and another from San Juan, Venezuela.

Measurements of an ordinary individual.—Length of body 0.098 mm.; width of body 0.053 mm.; diameter of nucleus 0.015 mm.; diameter of endospherule 0.0015 mm.; cilia line interval, anterior 0.00175 mm., posterior 0.0027 mm.

This form rather closely resembles *Zelleriella cusconis*, except that its nuclei in the resting condition are spherical. As the material from both infections is poorly preserved, the nuclei being often shrunken and evidently not normal, it is best to attempt no definite classification and to name it only provisionally.

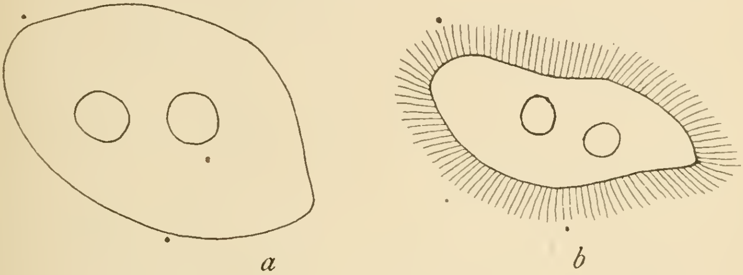


FIG. 77.—ZELLERIELLA [TRINITATIS], × 460 DIAMETERS.

ZELLERIELLA HYLAXENA, new species.

Type.—United States National Museum Cat. No. 16480.

Host.—*Hyla pulchella* Duméril and Bibron, one infection, in United States National Museum specimen No. 5407, 30 mm. long, from Paraguay; Captain Page, collector.

Measurements of an average individual.—Length of body 0.111 mm.; width of body 0.065 mm.; thickness of body 0.024 mm.; diameter of resting nucleus 0.0152 mm.; diameter of endospherule 0.002 mm.; cilia line interval, anterior 0.00155 mm., posterior 0.00285 mm.

The body of this species is unusually thick for a *Zelleriella*, its thickness being over one-third of its width. In the ordinary individuals the nuclei are spherical, or nearly so. In somewhat larger forms, preparing for division, the nuclei are a little elongated. In individuals ready for fission the two nuclei are dumb-bell-shaped with the constricted part of the dumb-bell narrow and rather long (fig. 78, *c*). Several daughter cells were seen with the two daughter nuclei pear-shaped and connected by a slender thread. It seems, therefore, that fission is consummated when the two nuclei are dumb-bell-shaped and much constricted. In figure 78, *c* is shown a large individual ready for fission. Each of its nuclei seems quite clearly to show four chromosomes in each end of the dumb-bell. But I am puzzled by another individual with a single *spindle-shaped* nucleus which lies tilted up at an angle of 45°. In this nucleus there is a ring of what are apparently macrochromosomes around the equator of the spindle, and their number seems quite clearly to be six. It seems that this must be abnormal, for a uninucleated individual should not, in this species, have a spindle-shaped nucleus. It should have either a much constricted dumb-bell nucleus, or, if older, two daughter nuclei either connected or not by a thread. Because of this peculiar nucleus one hesitates to say positively that there are four massive chromosomes in this species, yet apparently this is the number.

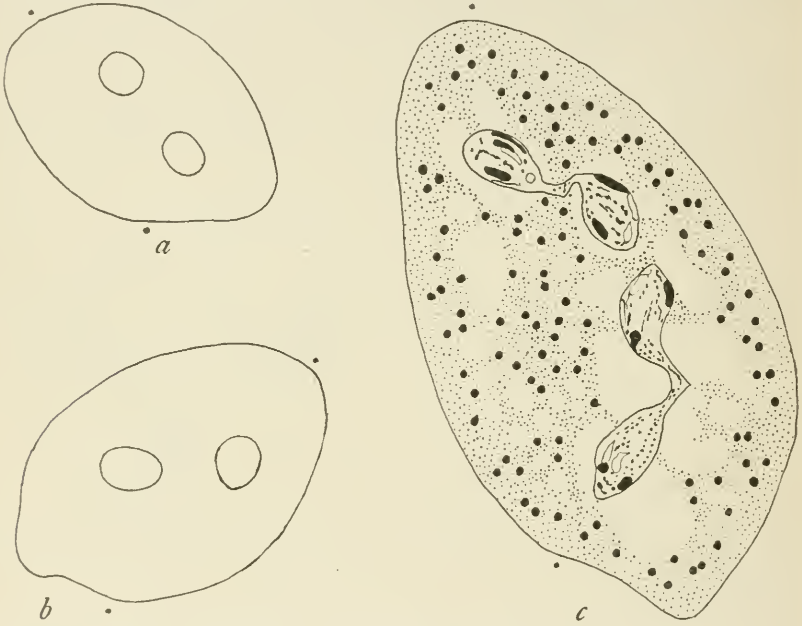


Fig. 78.—Zelleriella hylaxena, × 460 diameters: *a* and *b*, individuals of the ordinary sort; *c*, a a large cell ready for fission, the macrochromosomes on the under side of its nuclei drawn only in outline.

Zelleriella hylaxena resembles some individuals of *Z. patagoniensis*. The posterior point is developed to about the same extent, but the former species does not show any of the more wedge-shaped individuals such as are seen in *Z. patagoniensis*. The two species might perhaps be confused were it not that *Z. patagoniensis* has eight macrochromosomes while *Z. hylaxena* has apparently four, certainly not eight.

The animal shown in figure 78, *c*, is very highly vacuolated, the vacuoles being especially developed near the nuclei. Its condition is similar to that of a *Protoopalina intestinalis* which has been kept for a couple of days or so in salt solution and has its axial system of excretory vacuoles overemphasized.

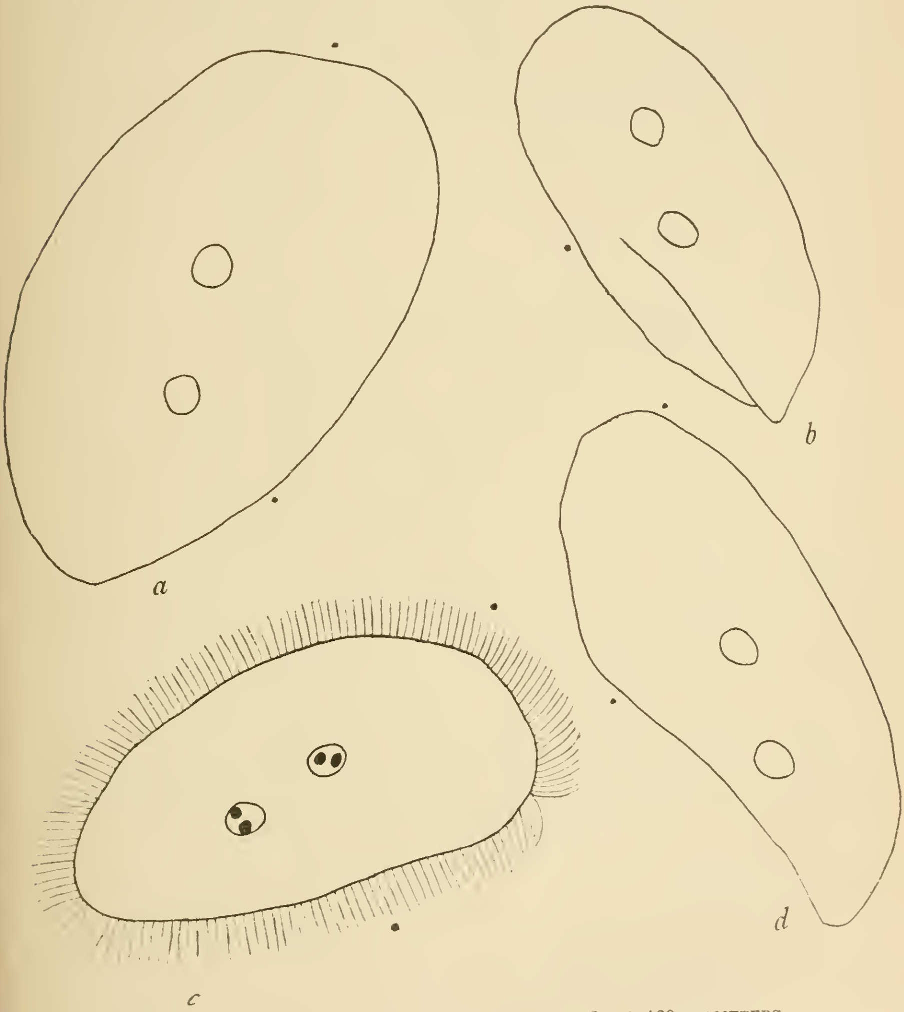


Fig. 79.—Zelleriella [of Hyla septentrionalis], × 460 diameters.

ZELLERIELLA [of HYLA SEPTENTRIONALIS], new species (?).

A specimen of this form has been deposited with the United States National Museum as Cat. No. 16481.

Host.—Hyla septentrionalis Boulenger, one abundant infection, in United States National Museum specimen No. 32029, 60 mm. long, from New Providence, Bahama Islands, June 19, 1903; "United States Fish Commission," collector.

Measurements of an ordinary individual.—Length of body 0.14 mm.; width of body 0.07 mm.; diameter of nucleus (somewhat shrunken) 0.0098 mm.; diameter of endospherule 0.0018 mm.; cilia line interval, anterior 0.00172 mm., middle 0.002 mm., posterior 0.0032 mm.

This somewhat wedge-shaped species has nearly spherical nuclei. Mitotic phenomena were not observed. The preservation is poor, the nuclei being shrunken with crinkled wall. Evidently in normal condition their diameter would be greater. The massive chromatin is generally aggregated into two or three balls of considerable size, almost surely an abnormal condition in this stage of the life-cycle.

Zelleriella [of *Hyla septentrionalis*] resembles *Z. brasiliensis* in form of body, but its resting nuclei are in a different mitotic phase, being more spherical than those of the latter species. It also resembles in form the more wedge-shaped individuals of *Z. couchii*, with which it agrees in all measurements except that its nuclei are but about two-thirds the size of those of *Z. couchii.* Since, however, the nuclei of *Z. septentrionalis* are shrunken, the two species may rather closely agree in the size of their nuclei. The average body form in the infection of *Z. septentrionalis* is different from the average body form in my material of *Z. couchii.* Their hosts belong to different families of Anura and one is found in the Bahamas, the other in Texas. More extensive and better preserved material from a larger series of infections in both hosts is needed for adequate comparisons, so I am merely referring the Bahama form to its host, leaving open the question of its validity as a species.

ZELLERIELLA VENEZUELAE, new species.

Type.—United States National Museum Cat. No. 16482.

Host.—*Hyla venulosa* (Laurenti), two scant infections, from La Guaira, Venezuela, July 3, 1900; Lyon and Robinson, collectors. Of these, the type infection was from United States National Museum specimen No. 27797, 95 mm. long.

Measurement: A, of a large, dividing individual; B, of an average individual—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.126	0.0935
Width of body	.0624	.06
Length of nucleus	.02	
Width of nucleus	.01	
Diameter of resting nucleus		.016
Diameter of endospherule	.0015	.0015

This species resembles *Z. hylaxena* in form of body and in size of nuclei. The individuals with resting nuclei are very similar and could hardly be distinguished, though the endospherules of the latter species are larger. But the nuclei of dividing individuals are very different. In *Z. hylaxena* fission is consummated when the two nuclei are each in a very constricted dumb-bell form, while in a similar fission stage of *Z. venezuelae* the nuclei are ellipsoidal and wholly unconstricted, a much earlier mitotic phase. As the nuclear phenomena are the most fundamental in the speciation of the Protoöpalinas and Zelleriellas, I do not hesitate to recognise *Z. venezuelae* as a distinct species, more archaic in its nuclear character than *Z. hylaxena*. It should naturally have been described before the latter species.

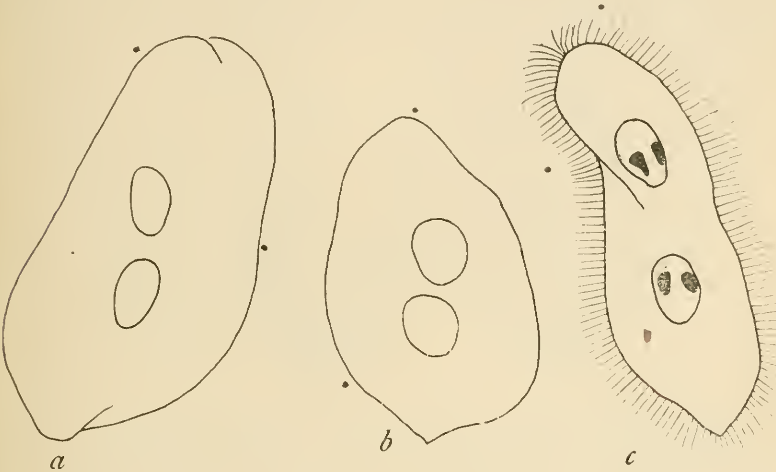


FIG. 80.—ZELLERIELLA VENEZUELAE, × 460 DIAMETERS: *c* SHOWS AN ALMOST EDGE VIEW OF THE ANTERIOR END AND AN OBLIQUE VIEW OF THE POSTERIOR END OF A TWISTED INDIVIDUAL.

ZELLERIELLA BINUCLEATA (Raff).

Opalina binucleata RAFF (1911).

Hosts.—Limnodynastes dorsalis (Gray) and *L. tasmaniensis* Guenther. both from Australia; Janet W. Raff, collector.

I have had no material of this species. Raff's description in full is as follows:

> *Opalina binucleata*, n. sp. This is found in great numbers in *Limnodynastes dorsalis* and on one occasion I met with it in *L. tasmaniensis*. It is a broad, flat form with two nuclei, and is ciliated equally over all its surface, the cilia being arranged in longitudinal rows as in other *Opalinae*. It is broader and more bluntly pointed at the posterior end than at the anterior [fig. 81*a*] and moves along with the anterior end foremost. Its usual position when swimming along is on either flat surface, but as it proceeds it occasionally rolls over from side to side. The average length is 157μ and the average breadth 100μ, but larger and smaller individuals have been met with. When the

animal turns over and presents itself edge on, it is seen to be very thin as compared with its breadth [fig. 81, *c*], and in section would appear flat or oval. Metcalf [1909] divides *Opalinae* into the following groups:

(1) Species with two nuclei, bodies circular in cross section.

(2) Species with many nuclei, body circular or broadly oval in cross section.

(3) Species with many nuclei, body flattened.

To these we may now add

(4) Species with two nuclei, body flattened. The nuclei measure 20μ across, and are circular in outline, and placed obliquely behind each other. The chromatin material is scattered about in masses and is not arranged in any definite order. There is no differentiation into ectosarc and endosarc visible from a general surface view, and the protoplasm appears vacuolated. During movement the posterior portion of the body shows a ridged or rucked appearance as indicated in figure 81, *b*, so that it seems to be contracted towards this end, and in this way it moves along.

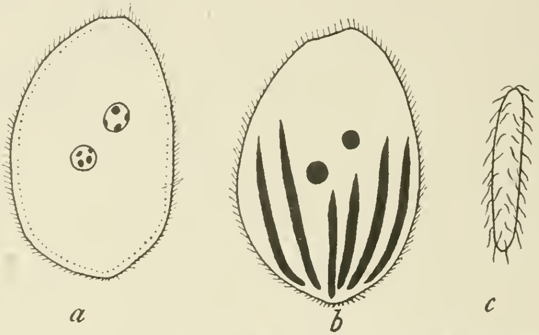


FIG. 81.—ZELLERIELLA BINUCLEATA. (AFTER RAFF.) THE HEAVY LINES IN *b* (SHADED IN RAFF'S DRAWING) REPRESENT "RIBS"; *c* IS AN EDGE VIEW.

Measurements of an ordinary individual.—Length of body, 0.157 mm.; width of body. 0.1 mm.; diameter of nucleus, 0.02 mm.

This species resembles in body form *Z. paludicolae* from *Paludicola brachyops* and *P. bibronii*, but has a more definite, though slight, posterior point. Its nuclei are doubtless seldom in mitosis or Raff would have described their appearance during this phase. The resting nuclei are considerably larger than in *Z. paludicolae*. It is in all likelihood a distinct species, though study of the mitosis would be desirable before definitely determining this point. *Z. binucleata* closely resembles in body from the shorter and broader daughter cells of *Z. brasiliensis* from *Leptodactylus ocellatus*, but in the latter species the nuclei are ellipsoidal in the resting condition.

THE ZELLERIELLAS OF THE BUFONIDAE.

The Zellerriellas found in the Bufonidae are peculiarly difficult to distinguish in some cases. We will first note the two species which have previously been described and will then pass to the numerous forms in my present material.

ZELLERIELLA MACRONUCLEATA (Bezzenberger).

Opalina macronucleata Bezzenberger (1904).

Host.—Bufo melanostictus Schneider, from "Asia." I have had no material of this species from this host, so quote data given by Bezzenberger.

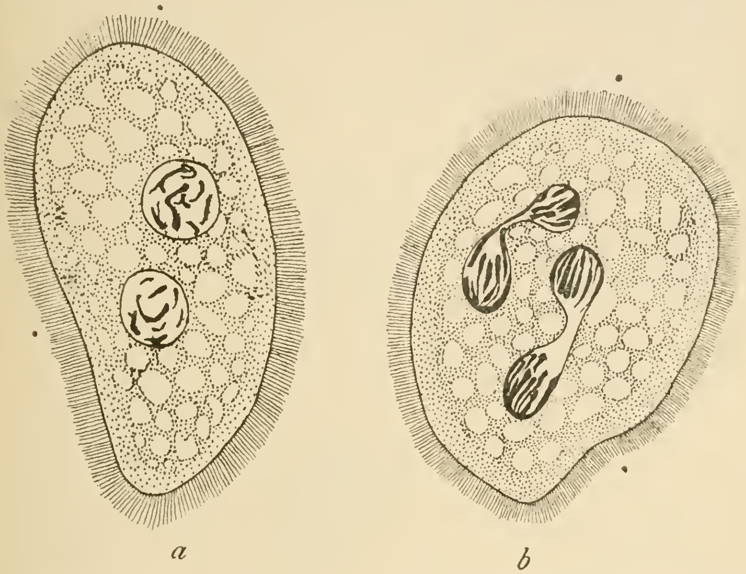


Fig. 82.—Belleriella macronucleata, × 952 diameters. (After Bezzenberger.)

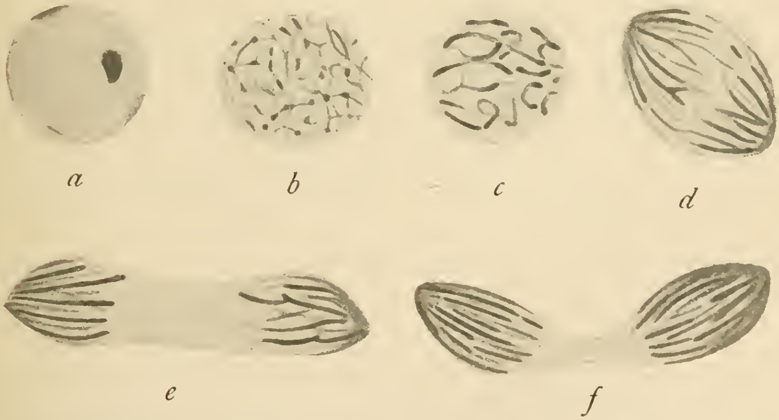


Fig. 83.—Zelleriella macronucleata, nuclei in different stages of mitosis, × about 2,000 diameters. (After Bezzenberger.)

Measurements of an ordinary individual with resting nuclei.—Length of body, 0.0631 mm.; width of body, 0.04 mm.; thickness of body (from fig. 14 in the text of Bezzenberger's paper), 0.0304 mm.; diameter of resting nucleus, 0.01204 mm.; length of cilia. 0.0042 mm.

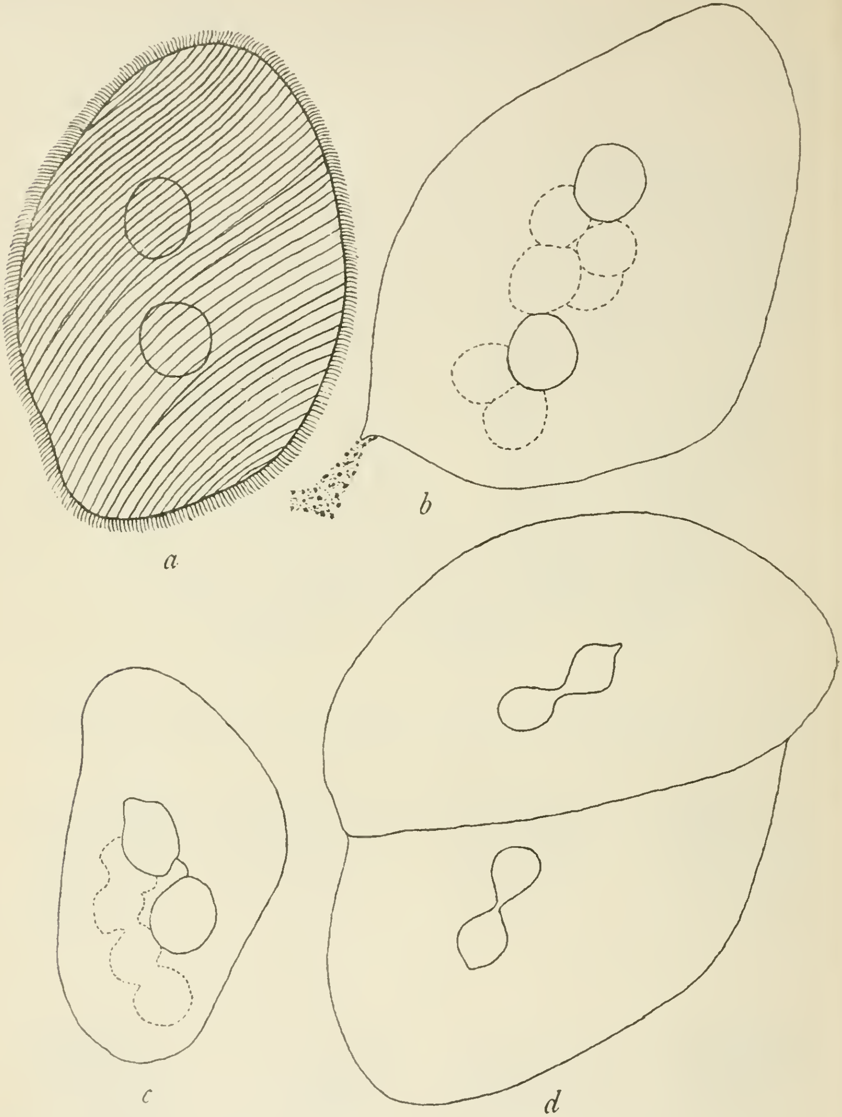


Fig. 84.—Zelleriella antilliensis, × 460 diameters: *a* and *b*, cells of the usual sort, *a* shows the lines of insertion of the cilia, *b* shows in dotted outline the axial excretory vacuoles, also posteriorly a trail of granules extruded from the excretory pore which evidently is situated at the base of a slightly developed posterior protuberance; *c* is a posterior daughter cell; *d* is in longitudinal fission.

The chromatin in the spherical, resting nucleus is in the form of "two or three large plaques at the surface of the nucleus," while "the rest of the nuclear contents show a finely reticulate structure." Bezzenberger's figures of stages of mitosis in unicellular [daughter] individuals are copied in figure 83. The macrochromosomes are not distinguished by Bezzenberger from the microchromosomes, his figures apparently showing only the former. If this be so, the number of macrochromosomes seems larger than in any other binucleated Opalinid now known, there appearing to be probably 12. (Fig. 83, *d* and *e*.) I regret that I have not material for restudy of the mitosis in this species.

In the section of this paper which discusses the geographical distribution of the Opalinids doubt is expressed of the assigning of this *Zelleriella* to *Bufo melanostictus* from "Asia." (See page 327.)

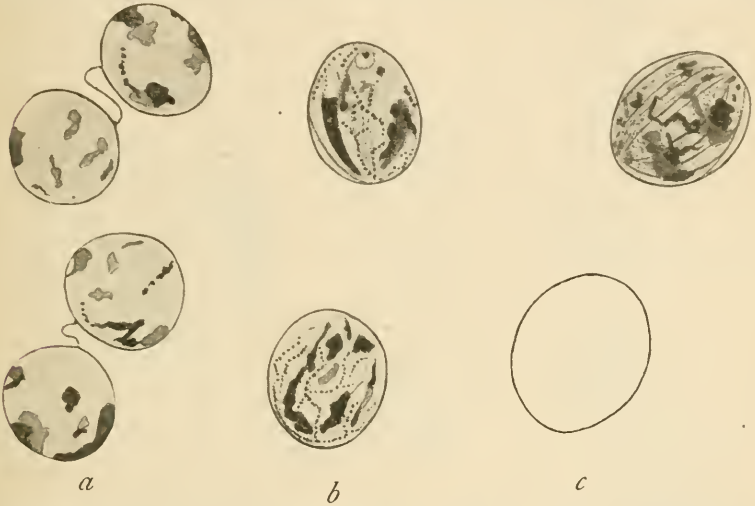


FIG. 85.—ZELLERIELLA ANTILLIENSIS, A SERIES OF NUCLEI, × 1,000 DIAMETERS: *a*, TWO PAIRS OF NUCLEI FROM A CELL IN LONGITUDINAL FISSION. (ONLY THE MACROCHROMATIN IS DRAWN. USUALLY FISSION IS COMPLETED BEFORE THE NUCLEI REACH THIS STAGE, DELAY IN THIS CASE BEING DUE TO KEEPING THE ANIMAL SEVERAL HOURS IN SALT SOLUTION); *b*, A PAIR OF NUCLEI FROM A LARGE CELL; AS IS FREQUENT, THE ANTERIOR NUCLEUS IS IN A SLIGHTLY MORE ADVANCED STAGE OF MITOSIS THAN THE POSTERIOR NUCLEUS; IT IS ALMOST IN THE CHARACTERISTIC IMPERFECT EQUATORIAL PLATE STAGE; THE POSTERIOR NUCLEUS IS PASSING OUT OF THE SKEIN STAGE, THE MACROCHROMOSOMES BEING NEARLY ALL DEFINED; SOME OF THE LINES OF MACROCHROMATIN GRANULES ARE DRAWN IN EACH NUCLEUS; *c*, A PAIR OF NUCLEI PASSING FROM THE EQUATORIAL PLATE STAGE INTO AN EARLY ANAPHASE. DETAIL IS SHOWN ONLY IN THE ANTERIOR NUCLEUS.

ZELLERIELLA ANTILLIENSIS (Metcalf) (figs. 84–86).

Opalina antilliensis METCALF (1914).

The type specimen of this species has now been deposited in the United States National Museum as Cat. No. 16483.

Host.—Bufo marinus (Linnaeus), from Jamaica, and from the Bermudas.

Measurements of an average individual with resting nuclei.—Length of body, 0.18 mm.; width of body, 0.113 mm.; thickness of body, 0.032 mm.; diameter of nucleus, 0.0278 mm.; diameter of endospherule, 0.00225 mm.; cilia line interval, anterior 0.002 mm., middle 0.0034 mm., posterior 0.00375 mm. Ten macrochromosomes, microchromosomes ten.

This is a much larger form than Bezzenberger's *Z. macronucleata* and it seems to have fewer macrochromosomes, judging from Bezzenberger's figures. It seems, however, a closely related species. Raff's *Z. binucleata* is also a similar form. A more detailed study of Raff's species, especially as to the nuclear conditions, might show them to be identical, though this is improbable in view of their geographical distribution and their residence in hosts which belong to different families of Anura.

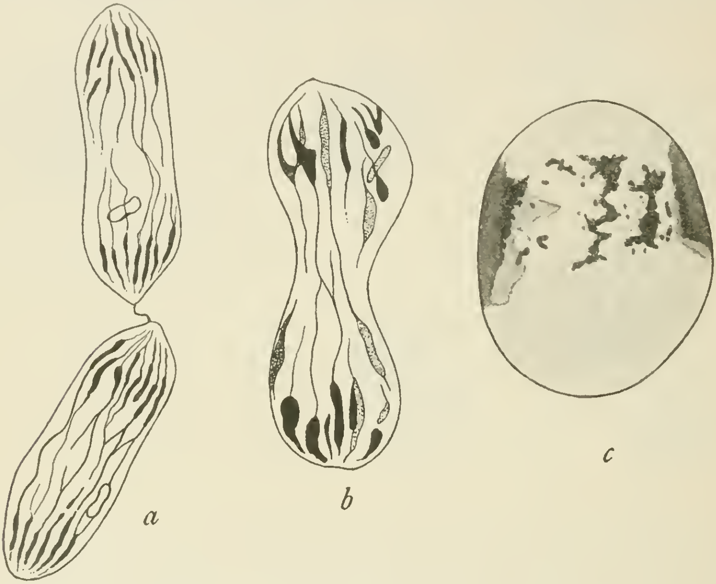


FIG. 86.—ZELLERIELLA ANTILLIENSIS, A SERIES OF NUCLEI: *a*, A PAIR OF NUCLEI FROM A CELL ENTERING UPON FISSION (EACH NUCLEUS IS IN A LATE ANAPHASE; ONLY THE MACROCHROMATIN IS DRAWN, AND THIS NOT ACCURATELY, THE NUMBER AND EXACT FORM OF THE MACROCHROMOSOMES NOT BEING SHOWN); *b*, THE POSTERIOR ONE OF A PAIR OF NUCLEI FROM A CELL IN LONGITUDINAL FISSION, SIMILAR TO THAT SHOWN IN FIG. 84, *d*, ONLY THE MACROCHROMATIN BEING DRAWN; *c*, ONE OF A PAIR OF NUCLEI FROM A DAUGHTER CELL, IN A VERY LATE TELOPHASE, THE CHROMOSOMES BEGINNING TO BREAK UP. SOME OF THE MICROCHROMATIC AND ACHROMATIC GRANULES ARE DRAWN, THE TWO SORTS NOT BEING DISTINGUISHED. *a* AND *b*, MAGNIFIED 1,000 DIAMETERS; *c*, MAGNIFIED 2,000 DIAMETERS.

Zelleriella antilliensis is the form in which attention was first drawn to the double series of chromosomes, there being 10 ribbon-shaped superficial macrochromosomes and 10 other more central microchromosomes, each consisting of a linear series of deeply staining granules larger than the achromatic granules of the nuclear stroma. Apparently this distinction between two types of chromosomes obtains throughout the Opalinidae. (See Loewenthal, 1908, also Metcalf, 1914.) Leger and Duboscq (1904, *b*) in their excellent description of *Protoopalina saturnalis* figure both macrochromosomes and microchromosomes (see fig. 37, *f*, p. 64 of this paper) but recognize as true chromosomes only the latter.

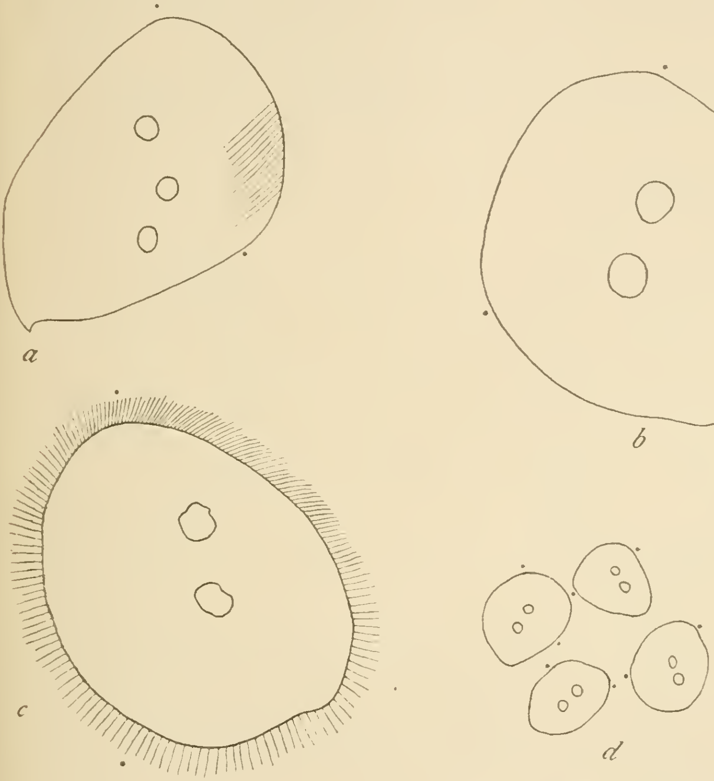


Fig. 87.—Zelleriella bufoxena: *a*, an apparently abnormal daughter cell with three nuclei, a portion of the lines of insertion of the cilia are drawn; *b*, an ordinary cell; *c*, an ordinary cell but with shrunken nuclei; *d*, four cells, × 117 diameters; *a*, *b*, and *c*, magnified 460 diameters.

ZELLERIELLA BUFOXENA, new species.

Type.—United States National Museum Cat. No. 16484.

Host.—*Bufo haematiticus* Cope, three scant infections, two very scant, from United States National Museum No. 14181 (a jar containing many toads, each of those found infected with this *Zelleriella* being 32 mm. long), from Nicaragua, August 7, 1885; J. T. Bransford, collector.

Measurements of an average individual.—Length of body 0.105 mm.; width of body 0.08 mm.; thickness of body of 0.013 mm.;

diameter of resting nucleus 0.0108 mm.; cilia line interval, anterior 0.00183 mm., posterior 0.00375 mm.

This is a very broad *Zelleriella* with nuclei much smaller than in *Z. macronucleata* or *Z. antilliensis.* One finds an occasional daughter cell with narrower form and a well-defined posterior curved point. In one such individual there were three very small nuclei (fig. 87, *a*), apparently an abnormal conditions which might conceivably arise in one of several ways not worth discussing without further data.

ZELLERIELLA MICROCARYA, new species.

Type.—United States National Museum Cat. No. 16485.

Host.—*Bufo lemur* Cope, two abundant infections, in United States National Museum specimens No. 27149 (the type infection) and No. 27150, each 73 mm. long, from Porto Rico, April 4, 1908; L. Stejneger, collector.

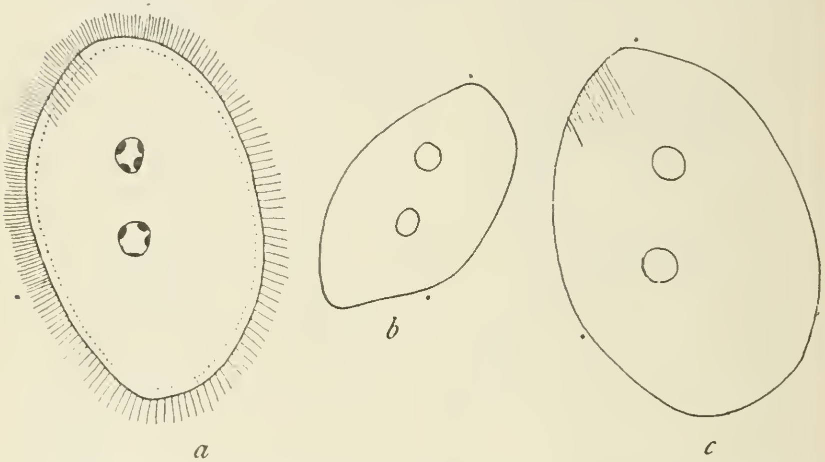


FIG. 88.—ZELLERIELLA MICROCARYA, × 460 DIAMETERS: *a* AND *c* SHOW ANTERIORLY A PORTION OF THE LINES OF INSERTION OF CILIA; *b* IS A DAUGHTER CELL.

Measurements of an ordinary individual.—Length of body 0.109 mm.; width of body 0.074 mm.; thickness of body 0.02 mm.; diameter of nucleus 0.00987 mm.; diameter of endospherule 0.0015 mm.; cilia line interval, anterior 0.0017 mm., posterior 0.004 mm.

This *Zelleriella* is of different form from *Z. bufoxena.* It is narrower and is evenly rounded posteriorly except in the case of the daughter cells from longitudinal fission, which in both species are wedge-shaped with a round-pointed posterior end. Like *Z. bufoxena* it has very small nuclei. Its nuclei are the smallest known among the binucleated Opalinids, being but little larger than the largest nuclei among the multinucleated species, as for example, *Opalina cincta.*

ZELLERIELLA INTERMEDIA, new species.

Type.—United States National Museum Cat. No. 16486.

Host.—*Bufo intermedius* Guenther, two abundant infections, from United States National Museum specimens No. 26161 and 26162, 22 mm. long (the type infection), both from Guanajuato, Mexico, A. Dugés, collector.

Measurements of an average individual.—Length of body 0.0937 mm.; width of body 0.05 mm.; thickness of body 0.016 mm.; diameter of resting nucleus 0.0109 mm.; length of endospherule 0.0015 mm.; width of endospherule 0.001 mm.; cilia line interval, anterior 0.0017 mm., middle 0.00285 mm., posterior 0.00225 mm. Four macrochromosomes.

This is a narrow, wedge-shaped species. One little peculiarity is observed, perhaps correlated with its form, namely, that the primary (major) lines of cilia are farther apart over the middle of the body than they are behind. The nuclei are nearly spherical, but not quite so. They are distinct from each other, not being connected by a thread. Each shows four distinct chromatin masses which seem to be macrochromosomes. These are arranged as in a very early metaphase, with the chromosomes distinct but irregularly placed.

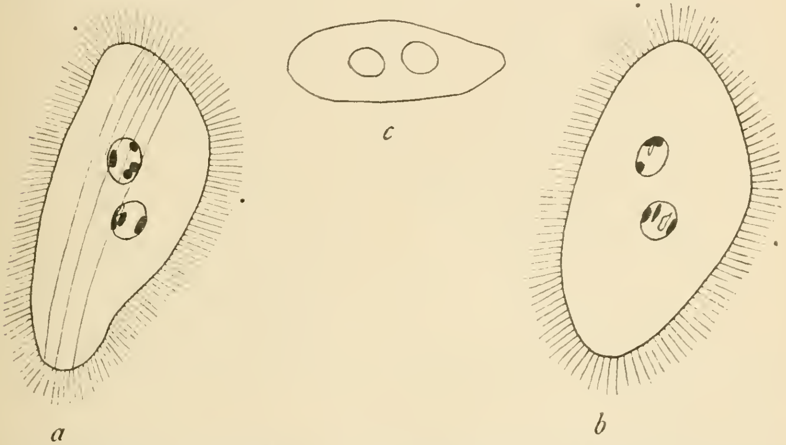


FIG. 89.—ZELLERIELLA INTERMEDIA, × 460 DIAMETERS: *a*, SHOWS SOME OF THE LINES OF INSERTION OF THE CILIA; *c* IS A VERTICAL, OBLIQUE, OPTICAL SECTION.

It seems appropriate to name this species *intermedia*, both because its host is *Bufo intermedius* and because in the size of its nuclei it is intermediate between *Z. bufoxena* and *Z. microcarya*, on the one hand, and some other species soon to be described.

ZELLERIELLA INTERMEDIA CUNEATA, new subspecies.

Type.—United States National Museum Cat. No. 16487.

Host.—*Bufo valliceps* Wiegmann, two abundant infections, from Brownsville, Texas, in United States National Museum specimens

No. 52279, 82 mm. long, the type infection, Jan. 5, 1915, and No. 52297, 57 mm. long, March 31, 1915, both collected by R. D. Camp.

Measurements of an average individual.—Length of body 0.1174 mm.; width of body 0.074 mm.; diameter of nucleus 0.0141 mm.; length of endospherule 0.0022 mm.; width of endospherule 0.0013 mm.; cilia line interval, anterior 0.00165 mm., middle 0.00287 mm., posterior 0.00375 mm.

The larger nuclei and the much larger, elongated endospherules in this form indicate that it is probably distinct, though it is very similar to the species type. Some larger individuals with ellipsoidal nuclei are found in one of the infections. These apparently are preparing for division. The spherical, resting nuclei show four chromatin masses of quite unequal size. The ellipsoidal nuclei are not well preserved for observation of internal structure.

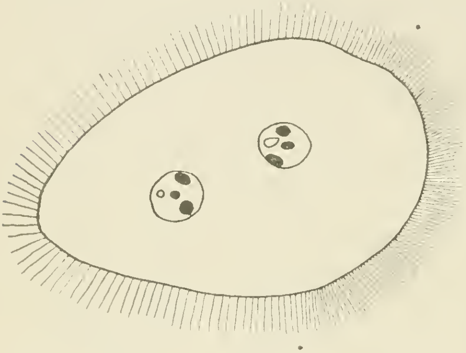


FIG. 90.—ZELLERIELLA INTERMEDIA CUNEATA, × 460 DIAMETERS.

ZELLERIELLA [of BUFO PELTOCEPHALUS], not described.

Host.—*Bufo peltocephalus* Tschudi.

One very scant infection along with *Protoopalina bufonis* in the United States National Museum in specimen No. 51864, 127 mm. long, from Cuba, June 3, 1914; J. B. Henderson and P. Bartsch, collectors.

The presence of these very few specimens of this *Zelleriella* was not detected until the stained material had faded too much for adequate study. No specimens are deposited in the National Museum.

ZELLERIELLA [of BUFO PUNCTATUS], new species (?).

A specimen has been deposited in the United States National Museum as Cat. No. 16488.

Host.—*Bufo punctatus* Baird and Girard, seven abundant infections. four from La Paz, California, and three from Furnace Creek, Death Valley, California, March 21, 1891. One of the latter, United States National Museum specimen No. 18769, 57 mm. long, a female with eggs 1 mm. in diameter, furnished the type infection.

Measurements: A, of a long, narrow individual; B, of a broad individual—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.132	0.1
Width of body	.063	.076
Diameter of nucleus	.0124	.0135
Cilia line interval:		
Anterior	.0023	....
Posterior	.0036	....

Dividing animals were not found in either infection, nor can internal nuclear structure be well studied. It seems best merely to label this form temporarily for reference until more satisfactory material is available for study.

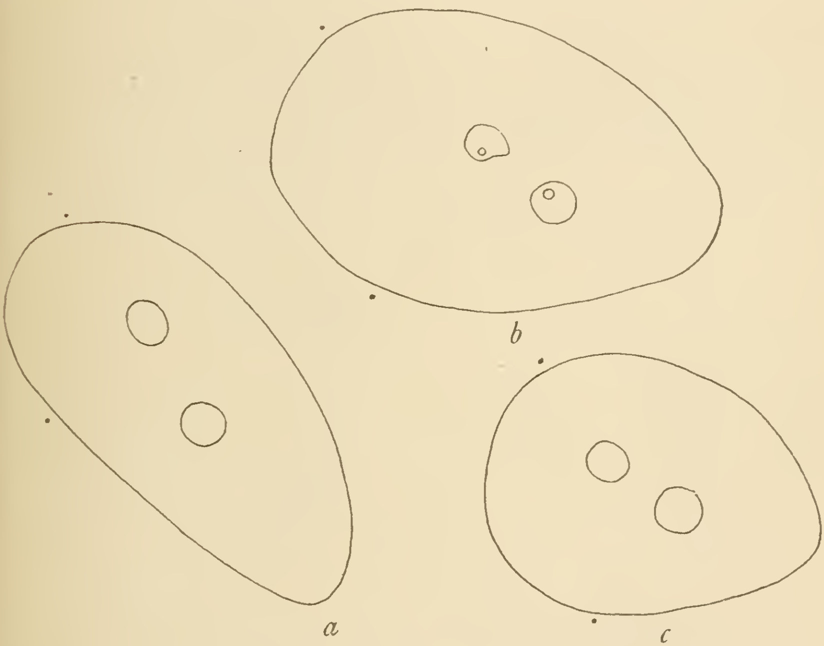


FIG. 91.—ZELLERIELLA [OF BUFO PUNCTATUS], × 460 DIAMETERS.

ZELLERIELLA [of BUFO STERNOSIGNATUS], new species (?).

A specimen of this form has been deposited in the United States National Museum as Cat. No. 16489.

Host.—Bufo sternosignatus Keferstein, three scant infections from Tehuantepec, Mexico, collected by F. Sumichrast. One of these, United States National Museum specimen No. 30436, furnished the type infection.

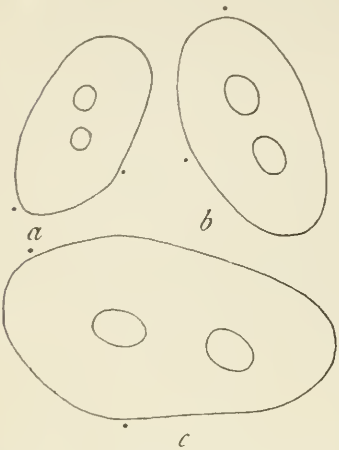


Fig. 92.—Zelleriella [of Bufo sternosignatus], × 460 diameters.

Measurements of a good-sized individual.—Length of body 0.098 mm.; width of body 0.053 mm.; length of nucleus 0.0152 mm.; width of nucleus 0.0108 mm.

These rather narrow Zelleriellas have more or less oval nuclei, except in the case of the small daughter cells, some of which show almost spherical nuclei. The preservation of my material is poor.

ZELLERIELLA [of BUFO SPINULOSUS], new species (?).

A specimen of this form has been deposited with the United States National Museum as Cat. No. 16490.

Host.—*Bufo spinulosus* Wiegmann, one abundant infection, in United States National Museum specimen No. 38575 (furnishing the type infection), 57 mm. long, from Lake Titi-

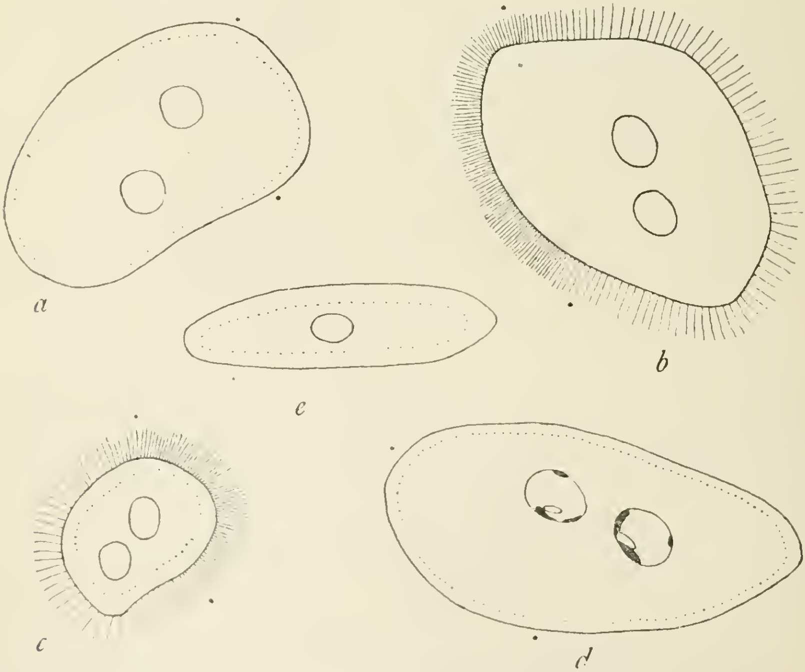


Fig. 93.—Zelleriella [of Bufo spindulosus], × 460 diameters; e is an optical, vertical section.

caca, Peru, July 31, 1908, R. E. Coker, collector, and one scant infection from La Paz, Brazil.

Measurements of an ordinary individual.—Length of body 0.1 mm.; width of body 0.0663 mm.; thickness of body 0.0237 mm.; length of nucleus 0.016 mm.; width of nucleus 0.012 mm.; length of endospherule, smallest 0.0016 mm., largest 0.025 mm.; width of endospherule, smallest 0.001 mm., largest 0.001 mm.; cilia line interval, anterior 0.0022 mm., posterior 0.0031 mm.

The slightly ellipsoidal nuclei were seen in several instances to contain four chromatin masses seeming to be macrochromosomes. Numerous cysts and a number of very small individuals were seen in the type infection, but nuclei in the more elongated conditions of mitosis were not observed.

ZELLERIELLA OPISTHOCARYA, new species (fig. 94).

Type.—United States National Museum Cat. No. 16492.

Host.—*Bufo coniferus* Cope, three very scant infections, two from Nicaragua, one, the type infection, from United States National Museum specimen No. 29976, 39 mm. long, from Costa Rica; Burgdorf and Schild, collectors.

Measurements: A, of a large individual; B, of a small form—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.326	0.142
Width of body	.138	.08
Thickness of body	.0285	....
Length of nucleus	.024	.0195
Width of nucleus	.0195	.0152
Cilia line interval:		
Anterior	.002	....
Posterior	.004	....

Characteristic of this very large *Zelleriella* is the position of its nuclei, far back in the body. The nuclei are slightly ellipsoidal, but the material is not sufficiently well preserved to allow study of their internal condition.

ZELLERIELLA OPISTHOCARYA [of BUFO MONXIAE] new species (?) (fig. 95).

A specimen of this form has been deposited with the United States National Museum as Cat. No. 16491.

Host.—*Bufo monxiae* Cope, two scant infections, one, the type infection, from United States National Museum specimen No. 11358, 29 mm. long, from Guanajuato, Mexico, January 30, 1880, A. Dugés, collector; the other infection from Yucatan.

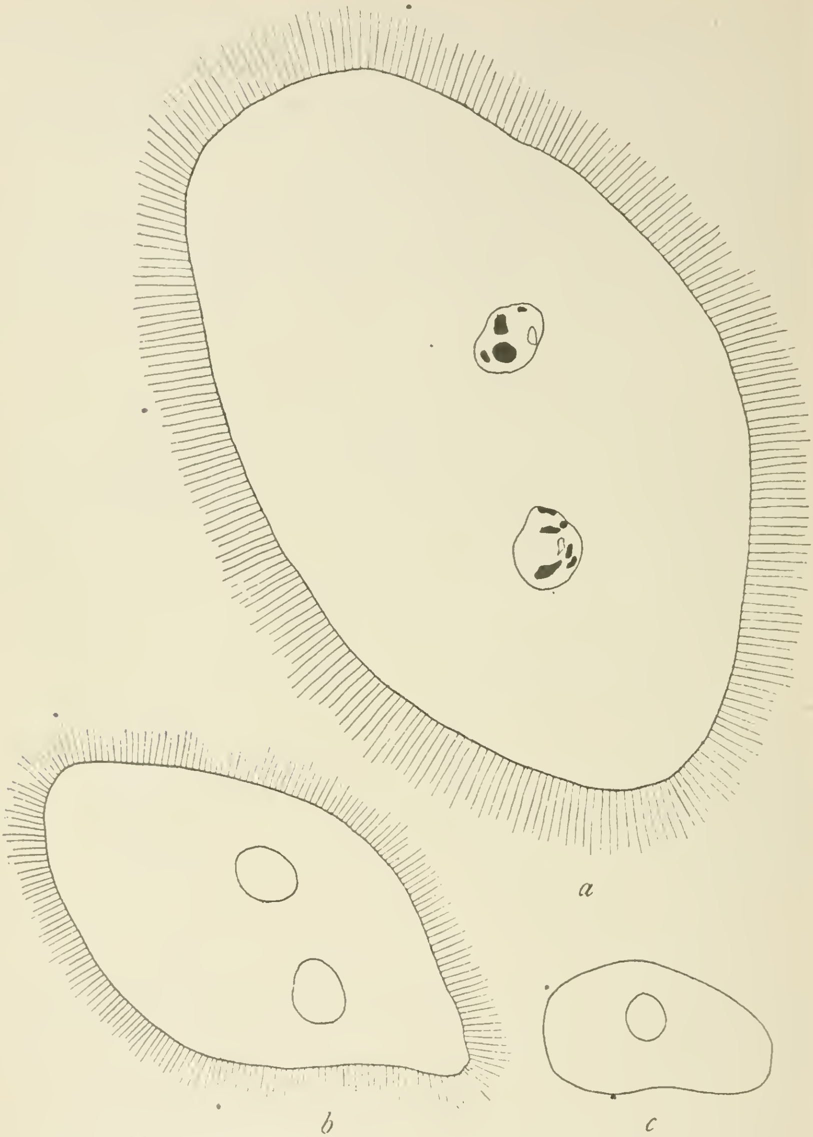


Fig. 94.—Zelleriella opisthecarya, × 460 diameters: *c* is a posterior (?) daughter cell from transverse (?) division.

Measurements of an ordinary individual.—Length of body 0.174 mm.; width of body 0.0956 mm.; thickness of body 0.02 mm.; diameter of shrunken nucleus 0.0152 mm.; diameter of endospherule 0.0015 mm.; cilia line interval, anterior 0.0014 mm., posterior 0.003 mm.

This good-sized *Zelleriella* has a rather characteristic form. Its spherical nuclei are shrunken. Around each nucleus in the cytoplasm, is a clear, narrow, empty space which seems as if it might have been filled by the nucleus before it shrank, but it may as well be the perinuclear portion of the excretory vacuole.

This form is perhaps identical with the one last described. The histological condition of the scant material available does not allow detailed study of the nuclei. These are more spherical than in typical *Zelleriella opisthocarya.* In both body form and spherical nuclei *Z. opisthocarya* [of *Bufo monxiae*] considerably resembles *Z. binghami*, a smaller species with its small nuclei usually well back in the body. The relationships are not clear without further study of a better series of well preserved infections. I am, therefore, provisionally merely referring this form from *Bufo monxiae* to its host as a possibly distinct variety of *Zelleriella opisthocarya.*

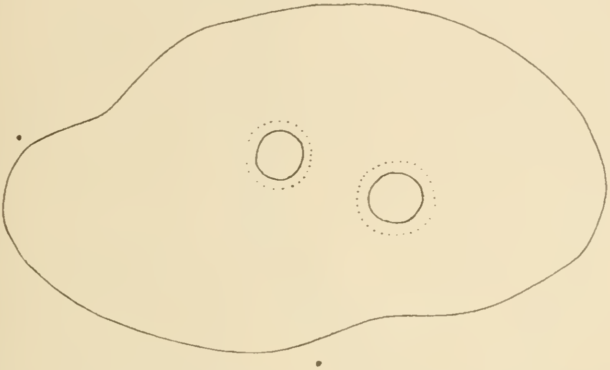


FIG. 95.—ZELLERIELLA OPISTHOCARYA [OF BUFO MONXIAE], × 460 DIAMETERS: THE DOTTED LINES AROUND THE NUCLEI INDICATE OUTLINES OF THE PERINUCLEAR VACUOLES.

ZELLERIELLA OPISTHOCARYA [of BUFO TYPHONIUS], new subspecies (?).

A specimen of this form has been deposited with the United States National Museum as Cat. No. 16493.

Host.—Bufo typhonius (Linnaeus), two abundant infections from Bahia Solada, Panama, J. F. Bransford, collector. The type infection is from United States National Museum specimen No. 25181, 17 mm. long.

Measurements: A, of a large individual; B, of a smaller form—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.17	0.114
Width of body	.12	.076
Length of nucleus	.0217	[a].0126
Width of nucleus	.0103	[a].009

[a] Shrunken.

This seems but a broader form of *Z. opisthocarya.* The large individuals for the most part have the nuclei placed well back in the body. The smaller individuals differ from one another, some showing the nuclei back, some having them farther forward. It seems that the former are anterior daughter cells, the latter posterior daughter cells. One posterior daughter cell was seen with its two nuclei well forward and still united by a thread. The material is not favorable for the study of the internal structure of the nuclei, though in the individual last mentioned an anaphase condition was evident, with seemingly eight macrochromosomes.

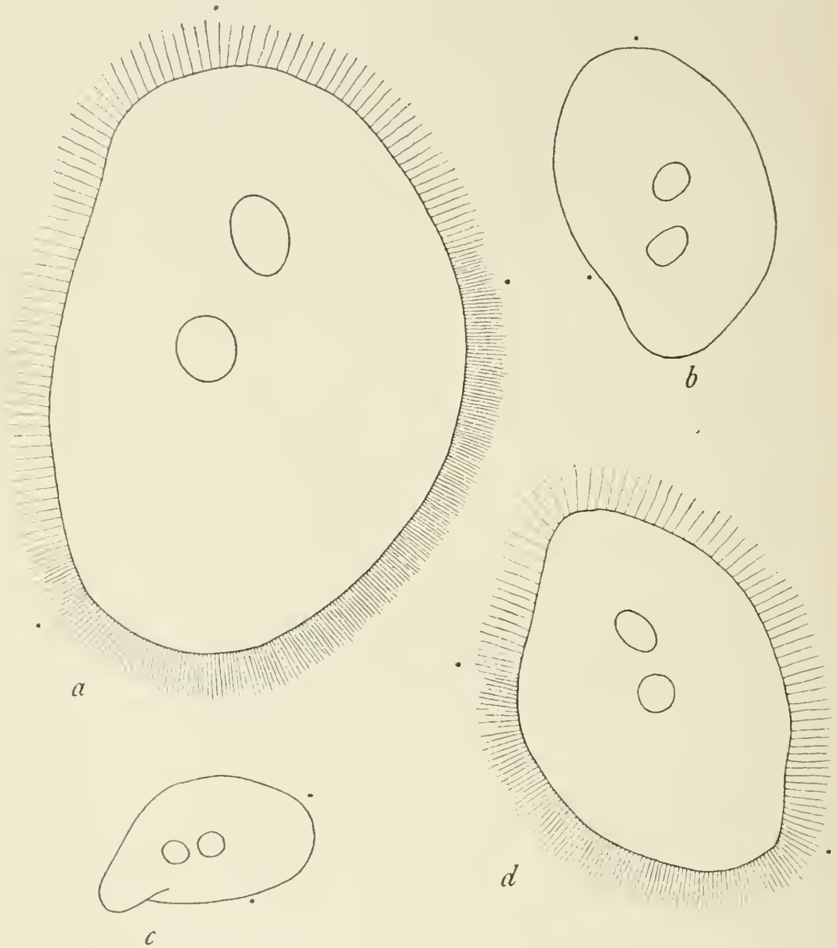


FIG. 96.—ZELLERIELLA OPISTHOCARYA [OF BUFO TYPHONIUS], × 460 DIAMETERS.

As I am unwilling, without further study than the material allows, definitely to assign this form to the species type, it is provisionally referred to its host as a possibly distinct subspecies.

ZELLERIELLA TELMATOBII, new species.

Type—United States National Museum Cat. No. 16498.

Host.—*Telmatobius jelskii* (Peters); two infections—one scant, from Guamote, Ecuador; the other, the type infection, from United States National Museum specimen No. 38577, 32 mm. long, from Blanca Island, Peru, July 23, 1908; R. E. Coker, collector.

Measurements of an ordinary individual.—Length of body, 0.191 mm.; width of body, 0.125 mm.; thickness of body, 0.024 mm.; length of nucleus, 0.023 mm.; width of nucleus, 0.0174 mm.: cilia line interval, anterior 0.00315 mm., middle 0.004 mm., posterior 0.0056 mm.

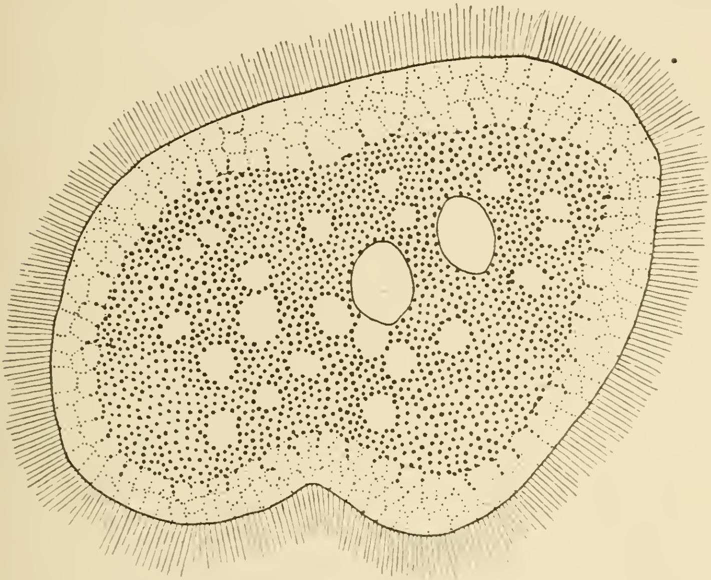


FIG. 97.—ZELLERIELLA TELMATOBII, × 460 DIAMETERS.

This large *Zelleriella* differs decidedly in body form from any other species of the genus, and, though much flattened, might almost be regarded as an aberrant *Protoopalina*. It seems much like *P. xyster*, except that instead of only the anterior part of the body being flattened, the whole body is flat.

The ectosarc in this species is very thick and is quite coarsely alveolated. The endosarc contains many large vacuoles, but is not so completely filled with them as is the endosarc of *P. xyster*. The nuclei are not well enough preserved for study of their internal structure, but in form and size they resemble those of *P. xyster*. The wide spacing of the cilia lines is a marked feature.

ZELLERIELLA [of BUFO WOODHOUSE] new species (?).

A specimen of this form has been deposited with the United States National Museum as Cat. No. 16499.

Host.—Bufo woodhousi Girard, one infection, from United States National Museum specimen No. 36364, 64 mm. long, from Utah, June 26, 1905.

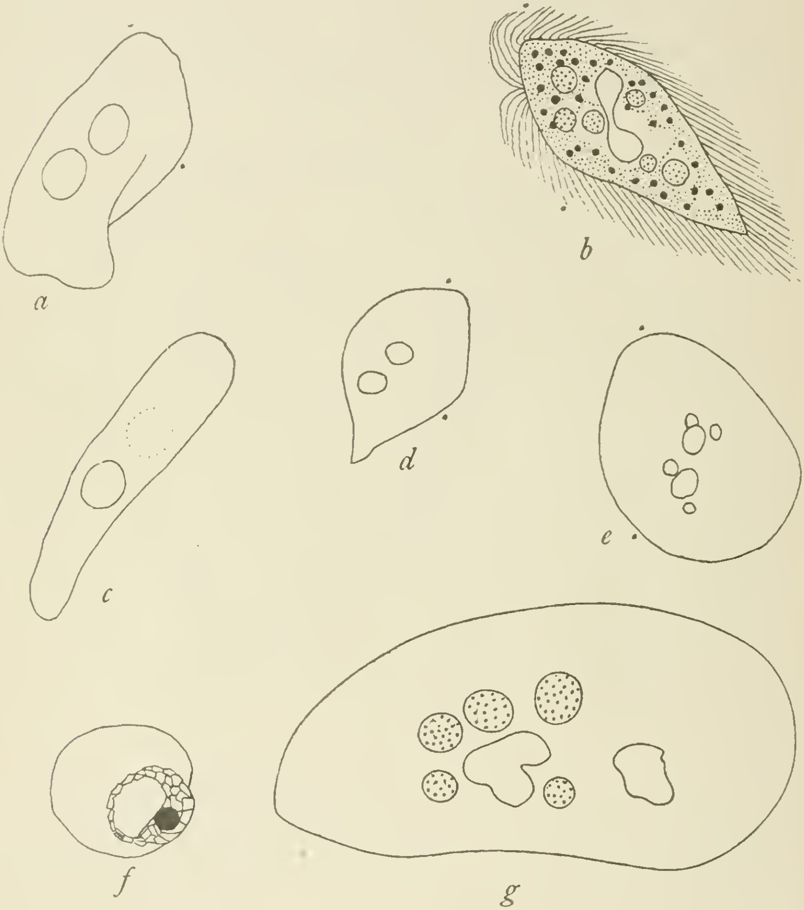


Fig. 98.—Zelleriella [of Bufo woodhousi]: *a*, a cell with two large nuclei; *b*, a daughter cell with one large dumb-bell nucleus and six small nuclei (?); *c*, an optical, sagittal section of a cell similar to that shown in *a*; *d*, a small cell with two small nuclei; *e*, a daughter cell from transverse division, with two large nuclei and four small nuclei (?); *f*, a degenerating nucleus; *g*, a large cell with two large irregular nuclei and five small nuclei (?) of different sizes; *f*, is magnified 1,000 diameters, the other figure 460 diameters.

Several different sorts of individuals are found in this very interesting infection. The figures show, 1, a large form with two large nuclei and numerous small "nuclei" of various sizes (*g*); 2, a good-sized individual with two large nuclei (*a*); 3, a daughter cell, recently come from longitudinal division, with one large dumb-bell nucleus and several small "nuclei" of varying sizes (*b*) (this significant individual will be discussed further); 4, a daughter cell from transverse division with two spherical daughter nuclei and four small "nuclei" (*e*); and 5, a small cell with two small nuclei (*d*). In this species and in *Z. hirsuta*, next to be described, we find phenomena of nuclear degeneration (?) which may be related to those described by Neresheimer (1907) for *Cepedea dimidiata* and *Opalina ranarum*. He reported the formation of abundant chromidia, the subsequent degeneration of the original nuclei, and the formation of new nuclei from the chromidia, these newly-formed nuclei persisting and functioning during the sexual phases of the life cycle.

I have not had living material for following these phenomena, and am in some doubt whether the conditions found in these two species of *Zelleriella* are really normal phenomena of the life cycle, as Neresheimer describes, or are abnormal, perhaps associated with parasites of the Zelleriellas. The latter seems the more probable. It is hardly profitable to discuss the matter at any length here, for I hope to obtain living material of one of the species, *Z. hirsuta*, for further study. A few words of comment, however, seem called for. Figure 98, *b*, shows a daughter cell with large nucleus in active mitosis, and also several small "nuclei" of varying sizes. Figure 98, *e*, shows a daughter individual which has passed into a little more advanced condition after fission, its two major nuclei being now distinct, but four other "nuclei," small, are present. We thus see that if the small nucleuslike bodies are true nuclei, formed from chromidia, as Neresheimer reports for two multinucleated species, the extrusion of the chromidia, which must have taken place some considerable time before, has not been followed as yet by degeneration of the original nuclei. These have persisted and have gone on dividing. On the contrary, when we come to the description of *Z. hirsuta* we will see that what appear to be the original nuclei do change their appearance in a way seeming to indicate their degeneration, and this is also true of the individual of the present species shown in figure *g*.

In my prolonged and detailed studies of *Protoopalina intestinalis* and *P. caudata* (Metcalf, 1909) I did not find these phenomena of apparent degeneration. I did find nuclear degeneration in *Opalina obtrigona*, but interpreted it as abnormal. The conditions for observation are so much better in binucleated Opalinids that it is important that these phenomena, if they occur, be studied thoroughly in them. At the same time the behavior of each of the two sets of chromosomes, massive and granular, should be observed in detail. The Zelleriellas are especially favorable for such study, for they have large nuclei and flat bodies, so that in smear preparations cytological detail can be seen almost as well as in sections. I hope to undertake this study upon *Z. antilliensis*, but anyone having favorable material of any *Zelleriella* would do well to take advantage of the opportunity. One of the species of *Zelleriella* with four chromosomes would be best.

If both the small and the large bodies in *Z.* [of *Bufo woodhousi*] are indeed true nuclei belonging to the Opalinid, we see that there may be much confusion in our data as to nuclear size if we are not careful, in our comparison of species, to compare similar phases of the life cycle, and the danger of confusion is equally great if the phenomena be abnormal.

Comparison with other Zelleriellas seems to indicate that *Z.* [of *Bufo woodhousi*] is a distinct species, though it is possible that some of the small Zelleriellas with small nuclei, already described, may be of this species, being taken at a time when their original nuclei have disappeared and been replaced by "secondary nuclei." Compare *Z. leptodactyli*, *Z.* [of *Hyla septentrionalis*], *Z. microcarya*. But I incline to the opinion that the phenomena of "secondary nuclei" are associated with parasitism and are abnormal.

Measurements: A, of a large individual with "degenerating primary nuclei and numerous secondary nuclei"; B, of a broad individual with "primary nuclei and four secondary nuclei"; C, of a daughter cell with one dumb-bell "primary nucleus and numerous secondary nuclei"; D, of a daughter (?) cell with two "secondary" (?) nuclei—

Measurements.	*A*	*B*	*C*	*D*
	mm.	*mm.*	*mm.*	*mm.*
Length of body	0.147	0.0685	0.085	0.0587
Width of body	0.074	0.0513	0.035	0.036
Diameter of:				
Nucleus	Collapsed.	0.0084	....	....
Largest secondary nucleus	0.013	0.004	0.0073	0.0076
Smallest secondary nucleus	0.0085	0.003	0.0048	....
Length of endospherule	0.002	....	....	....
Width of endospherule	0.0013	....	....	....
Cilia line interval	0.002	....	....	....

ZELLERIELLA HIRSUTA, new species.

Type.—United States National Museum Cat. No. 16500.

Host.—*Bufo cognatus* Say, two infections, one from Fort Mohave, Arizona, the other, the type infection, from United States National Museum specimen No. 35629, 64 mm. long, from Phoenix, Arizona; M. C. Dick, collector.

Measurements of an ordinary individual.—Length of body 0.113 mm.; width of body 0.06 mm.; thickness of body 0.0215 mm.; diameter of nucleus 0.011 mm.; diameter of largest "secondary" nucleus 0.0052 mm.; diameter of smallest "secondary" nucleus 0.003 mm.

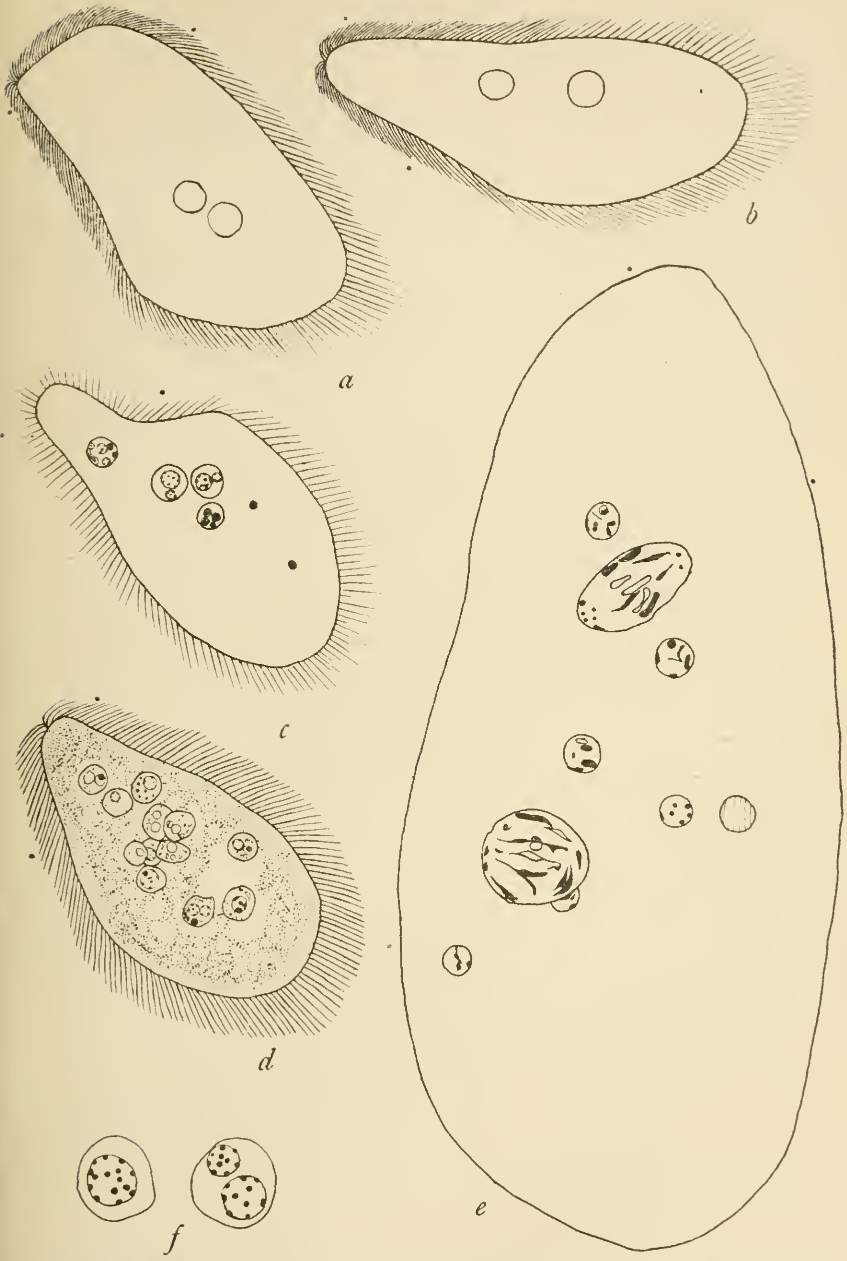


FIG. 99.—ZELLERIELLA HIRSUTA: *a* AND *b*, ORDINARY INDIVIDUALS; *c* AND *d*, INDIVIDUALS EACH WITH TWO DEGENERATING LARGE NUCLEI AND TWO (*c*) OR TEN (*d*) SMALL NUCLEI (?), IN *c* TWO ENDOSPHERULES (BLACK) ARE DRAWN; *e*, A CELL WITH TWO NORMAL NUCLEI IN MITOSIS AND SEVEN SMALL NUCLEI (?); *f*, TWO DEGENERATING NUCLEI FROM ONE CELL; NOTE THE ONE OR TWO SPHERES IN EACH, CONTAINING GRANULES OF CHROMATIN; *a* TO *d*, MAGNIFIED 460 DIAMETERS; *e* AND *f*, MAGNIFIED 1,000 DIAMETERS.

This *Zelleriella* is distinguished by the very dense ciliation, and the usually narrow form of its anterior end, and also by the position of the nuclei, which are usually well back in the body. The nuclear phenomena are similar to those in *Z.* [of *Bufo woodhousi*]. The "primary" nuclei, in my material, are readily distinguished by their darker staining, even in individuals in which they are not larger than the largest of the "secondary nuclei." The species seems quite distinct. Its body form and dense ciliation would almost justify classification in a distinct genus.

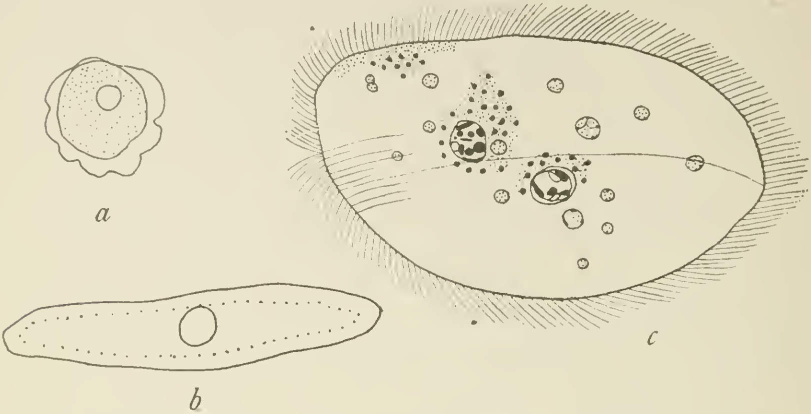


FIG. 100.—ZELLERIELLA HIRSUTA: *a*, A CYST, × 1,000 DIAMETERS; *b*, AN OPTICAL, VERTICAL SECTION, × 460 DIAMETERS; *c*, A CELL WITH TWO APPARENTLY NORMAL NUCLEI AND FIFTEEN SMALL NUCLEI (?) OF DIFFERENT SIZES, PORTIONS OF THE ENDOSARC WITH ITS ENDOSPHERULES BEING REPRESENTED, ALSO PORTIONS OF THE LINES OF INSERTION OF THE CILIA, × 460 DIAMETERS.

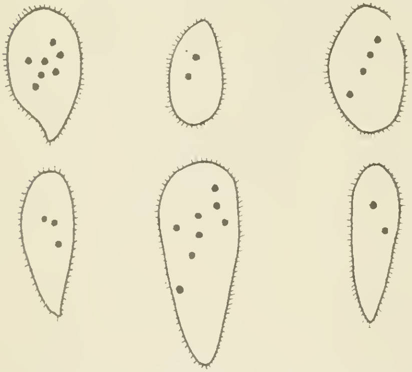


FIG. 101.—"ABNORMAL FORMS OF OPALINA" [ZELLERIELLA] FROM LIMNODYNASTES DORSALIS. (AFTER RAFF.)

In connection with the nuclear phenomena in this and the last described species it is interesting to note that Raff (1912) reports finding certain "abnormal" multinucleated forms (fig. 101), probably of *Zelleriella binucleata*, in a specimen of *Limnodynastes dorsalis* from Heathcote, Victoria, Australia. These individuals, of diverse shapes, had from three to eight nuclei arranged in no regular way. Raff regards them as "abnormal forms, in which the nuclei have divided irregularly without the body dividing." It seems likely that the phenomena observed were similar to those seen in *Zelleriella* [of *Bufo woodhousi*] and *Z. hirsuta.*

Subfamily Opalininae.

We have now come to the multinucleated forms. Of these there are two genera; *Cepedea*, circular, or nearly so, in cross section; and *Opalina*, much flattened. The *Opalinae* may be divided into two groups: *a*, species which are narrow and obovate, like *Opalina obtrigona*, and *b*, species which are broad, or even nearly round, as *Opalina ranarum*. *Cepedea* is named after Casimir Cepede, who has done extensive work upon the Ciliata Astomata and whose work has emphasized the fact that the old family Opalinidae (in the broader sense) is not a natural group, and that the old genus *Opalina* (including only my *Protoopalina*, *Zelleriella*, *Cepedea*, and *Opalina*) is quite distinct from the other Astomata, being in his usage the only genus in the family Opalinidae.

Among the Cepedeas we find considerable intergradation and many difficulties in demarcating species. This is even more true among the Opalinas. In these multinucleated forms the nuclear phenomena give us less assistance, for in many species the nuclei rest in the reticulate condition, as is characteristic of most other animals and plants. As already noted, the size of the nuclei varies with the physiological condition, and if there be really "primary" and "secondary" nuclei, these may differ in size. All we can do is to try to present the conditions found and express these in a taxonomic nomenclature which is admittedly tentative, until the life histories of the several species are known, and the races, present doubtless in all, or in most, species, have been studied.

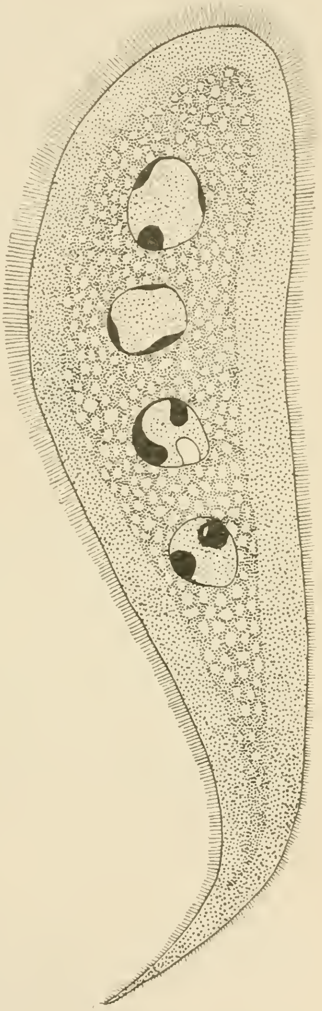


Fig. 102.—Cepedea lanceolata, × 1707 (?) diameters. (After Bezzenberger, 1904.)

Genus CEPEDEA.

CEPEDEA LANCEOLATA (Bezzenberger).

Opalina lanceolata Bezzenberger (1904).

Host.—*Rana esculenta*, variety *chinensis* Osborn, from "Asia."

I have had no material of this species, so quote from Bezzenberger's description.

Measurements.—Length of body 0.0822 mm.; width of body 0.0222 mm.: diameter of nucleus 0.007 mm.; length of cilia 0.0028 mm. These are the measurements given by Bezzenberger, but do not agree with his figures and his statement of the magnification of his figures. The magnification of figure 102 can hardly be "1707 diameters" and that of figure 103 be "350 diameters."

This *Cepedea* has its anterior end rounded and its posterior end elongated into a slender, tapering point. The nuclei are generally four in number, more rarely they are five. They are of uniform size and lie one behind the other in an axial row. The condition of the chromatin is shown in figure 102 and in figure 103, *a* to *e*. The number of macrochromosomes cannot be determined from Bezzenberger's figures, but seems to be unusually small for a *Cepedea*, also the nuclei are quite large. In both regards, in the size of its nuclei and the number of its macrochromosomes, *Cepedea lanceolata* is more like the Protoöpalinas than are other Cepedeas. It may well be a transitional species between the two genera.

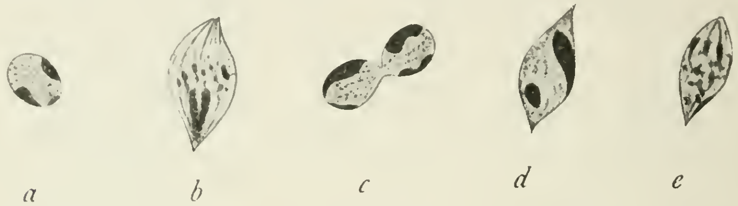


FIG. 103.—CEPEDEA LANCEOLATA; NUCLEI MAGNIFIED 350 (?) DIAMETERS, SHOWING DIFFERENT PHASES OF MITOSIS; (AFTER BEZZENBERGER): *a*, RESTING NUCLEUS; *b*, EQUATORIAL PLATE STAGE; *c*, DUMB-BELL DOUBLE METAPHASE; *d*, EARLY PROPHASE; *e*, ANAPHASE. THE INTERPRETATION OF THE STAGES OF MITOSIS IS NOT FROM BEZZENBERGER, BUT BY THE AUTHOR.

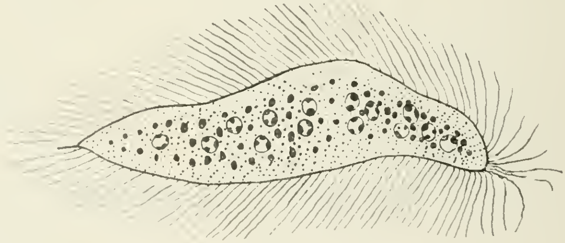


FIG. 104.—CEPEDEA SPINIFERA, × 460 DIAMETERS.

CEPEDEA SPINIFERA, new species.

Type.—United States National Museum Cat. No. 16501.

Host.—*Oxyglossus lima* Tschudi, one good infection from United States National Museum specimen No. 44022, 26 mm. long, from Buitenzorg, Java. March 6, 1909.

Measurements of an ordinary individual.—Length of body 0.124 mm.: width of body 0.03 mm.: length of posterior spine 0.0054 ± mm.: diameter of nuclei 0.0038 mm. to 0.0045 mm.; diameter of endospherule 0.00155 mm.: cilia interval, anterior 0.0018 mm., middle 0.00265 mm., posterior 0.00265 mm.

This *Cepedea* has the anterior end of the body slightly flattened, as is so usual with numerous species. The anterior end of the body is bent to one side, as is true of all cylindrical Opalinids, whether binucleated or multinucleated. The cylindrical species bend their bodies occasionally, as they move about, and an occasional preserved specimen may be found with a straight longitudinal axis, having been caught by the killing fluid and fixed in this temporary condition. Even the flattened forms, whether binucleated or multinucleated, show a similar bend in the front end of the longitudinal axis, as may be seen usually in the body form and especially in the course of the lines of cilia.

The posterior end of the body bears a very slender, spine-like tip from which the specific name is taken. The cilia in this species are very long, but it is unsafe to give any exact statement of the length from observation of preserved specimens, since the cilia are rarely perfectly preserved. The nuclei are spherical, or very slightly ellipsoidal, and are few in number, say from 10 to 18. The spheroidal endospherules are rather sparsely distributed through most of the body, but are much more crowded anteriorly. Crowding of the endospherules in the front end of the body is seen in some flattened forms. This species is quite sharply distinct from all others known.

CEPEDEA DIMIDIATA (Stein).

Opalina dimidiata Stein.

Hosts.—*Rana esculenta* Linnaeus; *Bufo vulgaris* Laurenti; *Bufo variabilis* Pallas, all from Europe.

Specimens of this Opalinid have been deposited with the United States National Museum as Cat. Nos. 16506 to 16509.

This well known *Cepedea* shows great diversity in size in different infections and as great diversity in the proportions of the body. Some are slender and some very stocky, the most stocky individuals being among the largest of the Opalinidae. These largest, stockiest individuals have been treated by Neresheimer (1907) and Metcalf (1909) as a distinct species, *zelleri*, but further study of a large number of infections shows a complete series of intergrading forms. Sometimes in a single infection there will be found quite slender forms and very stocky individuals and numerous intergrades (fig. 105). It seems, therefore, that we have here, as in *Protoopalina caudata*, *P. saturnalis*, and a number of other species of all four genera of Opalinids, merely a slender and a stocky form of the one species, with intergrading individuals.

Cepedea dimidiata shows the usual characteristics of the Cepedeas, the body being somewhat spindle-shaped, with the anterior end not much narrowed but bent to one side, while the posterior part of the body tapers to a rounded point. The endospherules are usually more numerous anteriorly. The very small individuals in the spring tend to be more slender, especially their posterior ends, and the young forms after sexual reproduction, that is, zygotes which have multiplied the number of their nuclei to say four or more, are generally extremely slender and tapering, almost as much so as a microgamete mother cell. The nuclei are almost all spherical. A few will be found in active mitosis. The number of macrochromosomes seems to be 12.

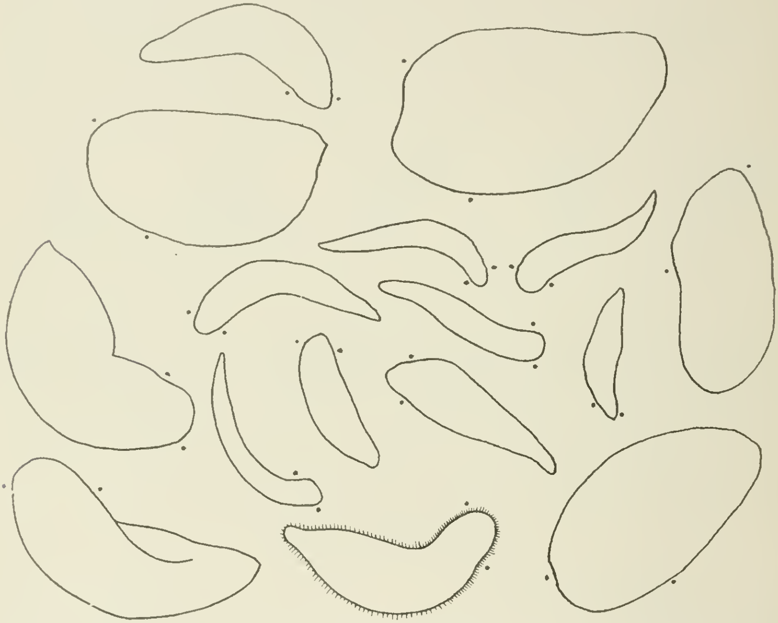


Fig. 105.—Cepedea dimidiata and its form zelleri, × 117 diameters. The outer eight figures represent the zelleri form. All the individuals drawn are from a single individual of Rana esculenta.

SLENDER FORM.

See United States National Museum Cat. Nos. 16506 and 16507.

Measurements of an average individual, of the slender form, from Rana esculenta.—Length of body 0.18 mm.; width of body 0.0256 mm.; diameter of nucleus 0.004 mm. to 0.0045 mm.; length of endospherule 0.0022 mm.; width of endospherule 0.00125 mm.; cilia line interval, anterior 0.0028 mm., middle 0.003 mm., posterior 0.0033 mm.

CEPEDEA DIMIDIATA, form ZELLERI (i. e., stocky form).

See United States National Museum Cat. No. 16508.

Measurements of a large individual from Rana esculenta.—Length of body 0.32 mm.; width of body 0.184 mm.; diameter of nucleus 0.0056 mm. to 0.00765 mm.; length of endospherule 0.0025 mm.; width of endospherule 0.002 mm.; cilia line interval, anterior 0.0012 mm., posterior 0.003 mm.

The posterior ends of the largest individuals are not tapered, but broadly rounded, and are often thrown into rounded ridges. Yet often in these stockiest individuals a slight point may be seen, corresponding to the posterior point, "spine," or "tail" seen in numerous other species. The total number of nuclei in one of the largest of these stocky individuals is about 200.

INTERMEDIATE FORMS.

See United States National Museum Cat. No. 16509.

Intermediate forms completely intergrading between the most slender sort, on the one hand, and the stockiest sort, on the other, are common, and this intergradation is shown not only in size and form of body, but in size of nuclei as well. If this were a rare and inaccessible species and say only two infections were known, one of slender individuals and the other of the *zelleri* sort, no one would for a moment consider placing them both in the same species. This illustrates well the necessarily tentative nature of taxonomic conclusions based upon anything less than a complete series of infections, showing individuals from all phases of the life conditions and the life cycle. An intensive study of a few species from each genus of the Opalinidae is a desideratum, with a view to determining racial differences and structural diversities and their correlation with physiological conditions, or with phases of the life cycle.

CEPEDEA DIMIDIATA ORIENTALIS, new subspecies (fig. 106).

Type.—United States National Museum Cat. No. 16511.

Host.—*Rana nigromacultata* Hallowell, two infections, one scant; the other, the type infection, very abundant, from United States National Museum specimen No. 23579, 61 mm. long, from Yokohama, Japan, September, 1896, L. Stejneger, collector.

Measurements of a medium-sized individual.—Length of body 0.18 mm.; width of body 0.045 mm.; diameter of nucleus 0.00375 mm. to 0.004 mm.; diameter of endospherule 0.0016 mm. to 0.0018 mm.; cilia line interval, anterior 0.002 mm., posterior 0.0035 mm.

This *Cepedea* is similar to *C. dimidiata*, but in my infections the slender individuals are less slender and the broader individuals more slender than are the corresponding forms of the species itself. In other words the two extremes are much less divergent in the subspecies *orientalis* than they are in *C. dimidiata* proper. The cilia are longer than in *C. dimidiata.* The broader individuals are often spirally ridged in a peculiar way shown in figure 106, *a.* The Japanese forms might about as well be recognized as a distinct species.

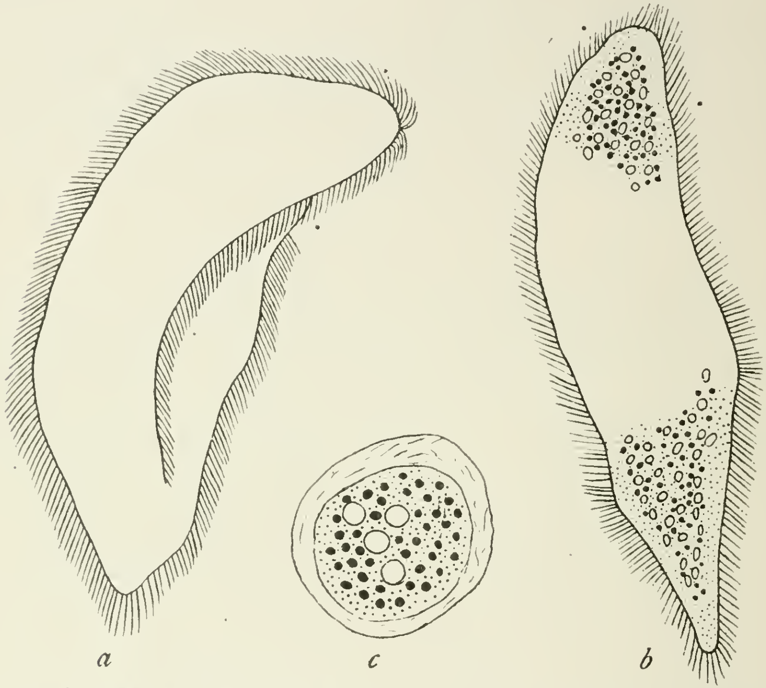


Fig. 106.—Cepedea dimidiata orientalis: *a*, an outline drawing of a stocky individual, × 460 diameters; *b*, an optical section of a more slender individual from the same infection, × 460 diameters; *c*, a cyst, × 1,000 diameters.

CEPEDEA DIMIDIATA [PARAGUENSIS], new subspecies (?).

A specimen of this form has been deposited with the United States National Museum as Cat. No. 16526.

Host.—Hyla nassica Cope, one scant infection, from United States National Museum specimen No. 6226, from Paraguay; Captain Page, collector.

Dimensions of an ordinary individual.—Length of body 0.1382 mm.; width of body 0.0456 mm.; diameter of nucleus 0.003 mm. to 0.0044 mm., mean 0.004 mm.; cilia line interval, anterior 0.0019 mm., posterior 0.00335 mm.

This *Cepedea* is quite similar to *C. dimidiata orientalis.* It is hard to describe any diagnostic difference betwen them. They may be identical, but the great divergence in their geographical habitats and their residence in hosts of different families make this very improbable. It seems best to give the Paraguayan form a provisional subspecific name for convenience of reference, pending further study.

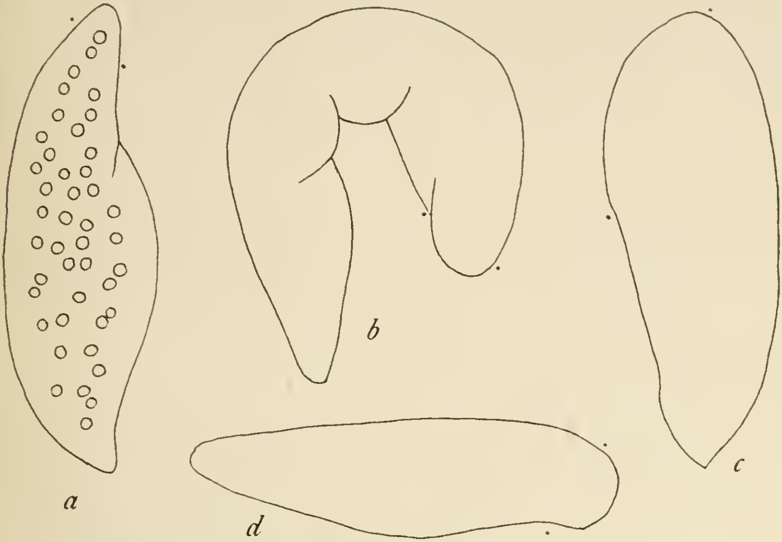


FIG. 107.—CEPEDEA DIMIDIATA [PARAGUENSIS], × 460 DIAMETERS.

CEPEDEA DIMIDIATA HAWAIENSIS, new subspecies.

Type.—United States National Museum Cat. No. 16512.

Host.—*Rana catesbeiana* Shaw, numerous infections from bullfrogs purchased from a San Francisco, California, dealer, who said the frogs were from Hawaii, having been originally introduced into Hawaii some years before from North America. I have opened over 40 bullfrogs (this species), large and small, from the eastern and the southeastern United States and have never found an infection of *Opalina*. I have opened also many bullfrog tadpoles, of different ages, with a similar result. On the other hand a considerable proportion of these Hawaiian bullfrogs purchased in San Francisco showed good infections.

Measurements: A, of a large, spirally twisted individual; B, of a somewhat smaller individual—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.2	0.17
Width of body	.06	.043
Length of nucleus	.006	.0065
Width of nucleus	.0044	.0057
Cilia line interval, middle	....	.0025
Cilia short, like those of *C. dimidiata*.		

The smaller individuals of this Hawaiian *Cepedea* are shaped as are ordinary *C. dimidiata*. Many of the larger individuals, on the other hand, are spirally ridged, presenting a very characteristic appearance (fig. 108, *b*). Intermediate forms show a condition much like the larger forms of *C. dimidiata orientalis*. The nuclei are ellipsoid and quite large. The cilia are short as in other *dimidiata* forms.

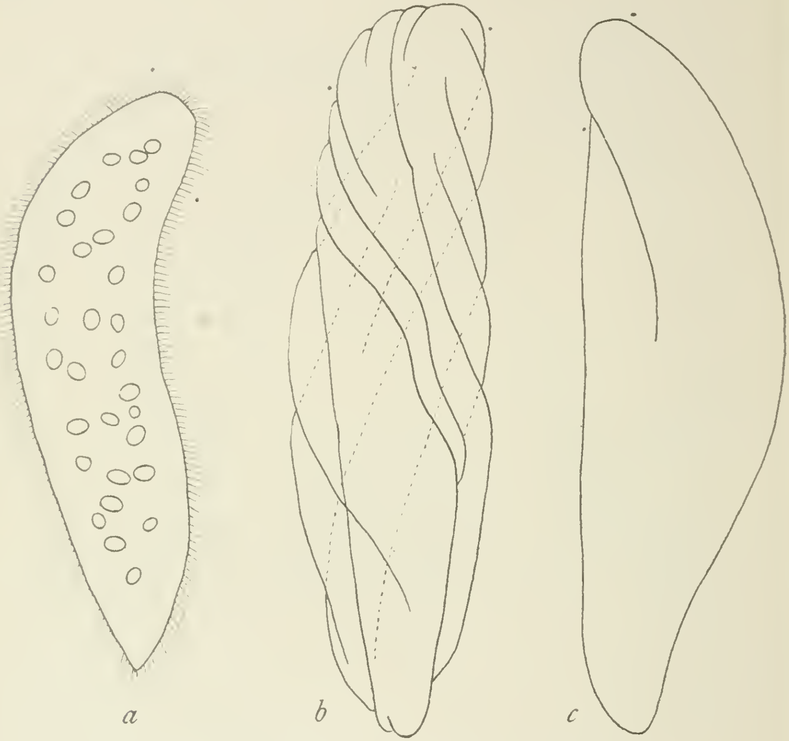


Fig. 108.—Cepedea dimidiata hawaiiensis, × 460 diameters.

CEPEDEA SAHARANA, new species.

Type.—United States National Museum Cat. No. 16620.

Host.—*Rana esculenta ridibunda* (Pallas), three abundant infections and one scant infection from Biskra, Algeria, collected May 26, 1912. by D. D. Streeter, jr. The type infection is from United States National Museum specimen No. 49840.

Measurements of a large, Paramecium-shaped individual.—Length of body 0.245 mm.; width of body 0.057 mm.; diameters of nuclei from 0.0027 mm. to 0.003 mm.

Measurements of a small, more stocky individual.—Length of body 0.104 mm.; width of body 0.048 mm.; diameters of nuclei the same as in the more slender individuals.

This *Cepedea* has something the shape of *C. dimidiata*, but its more slender individuals are less slender than the slenderer form of the latter species, and its most stocky individuals are much less stocky than the most pronounced *zelleri* form of *C. dimidiata*, but a larger series of infections might very likely show greater divergence of form than do my four infections taken at one time from one locality. Many individuals have a form much resembling that of *Paramecium* (Fig. 109, *d* and *b*). The species is distinguished from *C. dimidiata* by its minute nuclei. In both the slender and the broader individuals the nuclei are but from 0.0027 mm. to 0.003 mm. in diameter, while the nuclei of the slender form of *C. dimidiata* are about five-thirds as large, and those of the form *zelleri* are from twice to three times as large.

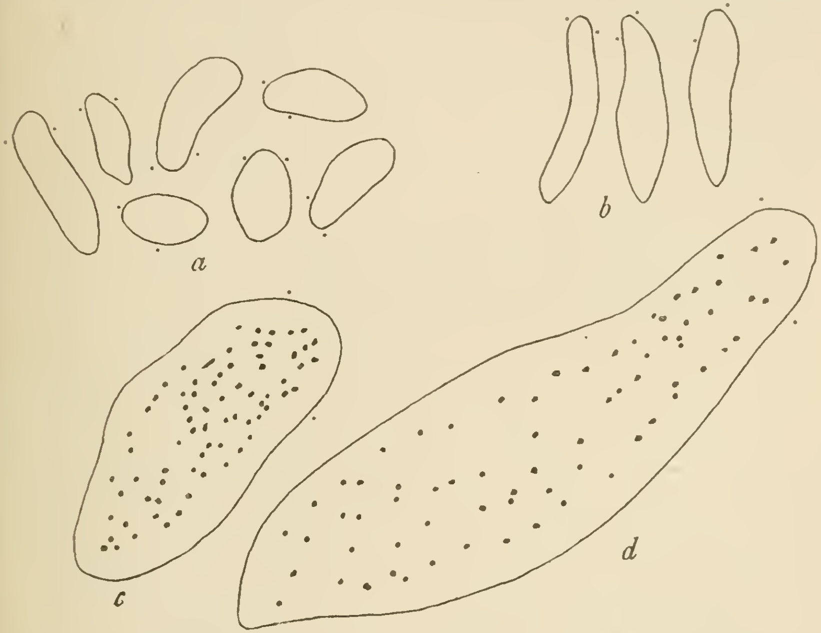


Fig. 109.—Cepedea saharana: *a*, a group of animals, showing the range of size and form in a single infection, × 117 diameters; *b*, three characteristic individuals from another infection, × 117 diameters; *c*, an individual from the same infection as *a*, magnified 460 diameters; *d*, an individual from the same infection as *b*, magnified 460 diameters. In *c* and *d* all the nuclei are indicated, there being 71 in *c* and 72 in *d*. The ratio between the bulk of the body and the combined bulk of all the nuclei is evidently not the same in *c* and *d*.

CEPEDEA BUERGERI, new species.

Type—United States National Museum Cat. No. 16582.

Host—*Polypedates buergeri* (Schlegel), three infections from Japan, the type infection being from United States National Museum specimen No. 23904, 42 mm. long, from Province Iga, Hondo, Japan; I. Ijima, collector.

Measurements: A, of a large individual; B, of a smaller individual of the same shape; C, of a slender individual—

Measurements.	*A*	*B*	*C*
	mm.	*mm.*	*mm.*
Length of body	0.2	.161	0.213
Width of body	.106	.065	.06
Thickness of body	.0305	.035	....
Diameter of nucleus	.0048– .0055	.0042– .006	....
Diameter of endospherule	....	.0016– .00175	....
Cilia line interval:			
Anterior	.0021	.0016	....
Middle	.0039	.00375	....
Posterior	.0045	.0038	....

This *Cepedea* resembles a somewhat flattened *C. dimidiata.* It has individuals of stocky and of slender form, the latter, however, are not pointed posteriorly, as are some of the most slender *C. dimidiata.* The thickness of the body is from one-third to one-half, or more, as great as the width. It seems, therefore, somewhat intermediate between the Cepedeas and the Opalinas.

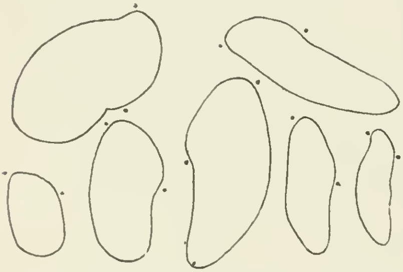


FIG. 110.—CEPEDEA BUERGERI: A GROUP OF CELLS FROM A SINGLE INFECTION, × 117 DIAMETERS.

CEPEDEA BUERGERI SINENSIS, new subspecies.

Type.—United States National Museum Cat. No. 16589.

Host—*Bufo gargarizans* Cantor, one infection, from United States National Museum specimen No. 46490, from Hong Kong, China, 1883; P. L. Jouy, collector.

Measurements: A, of a large individual; B, of a smaller form—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.211	0.16
Width of body	.1022	.0815
Thickness of body	.04	.042
Diameter of nucleus	.0055– .0065	.0045
Cilia line interval, anterior	....	.002

This *Cepedea* is about half as thick as wide, seeming intermediate between the Cepedeas and the Opalinas. Considered as a flat form, it is intermediate between the *obtrigona*-like species and the *ranarum*-like species, being, on the whole, more *ranarum*-like. It suggests comparison with *Cepedea buergeri* and is seemingly a nearly related form. If my material contained any slender individuals, such as are seen in *C. buergeri*, one would refer this infection to the species proper, but as the forms studied are all broader I am classing them as a subspecies of *C. buergeri*. A fuller series of infections is a desideratum.

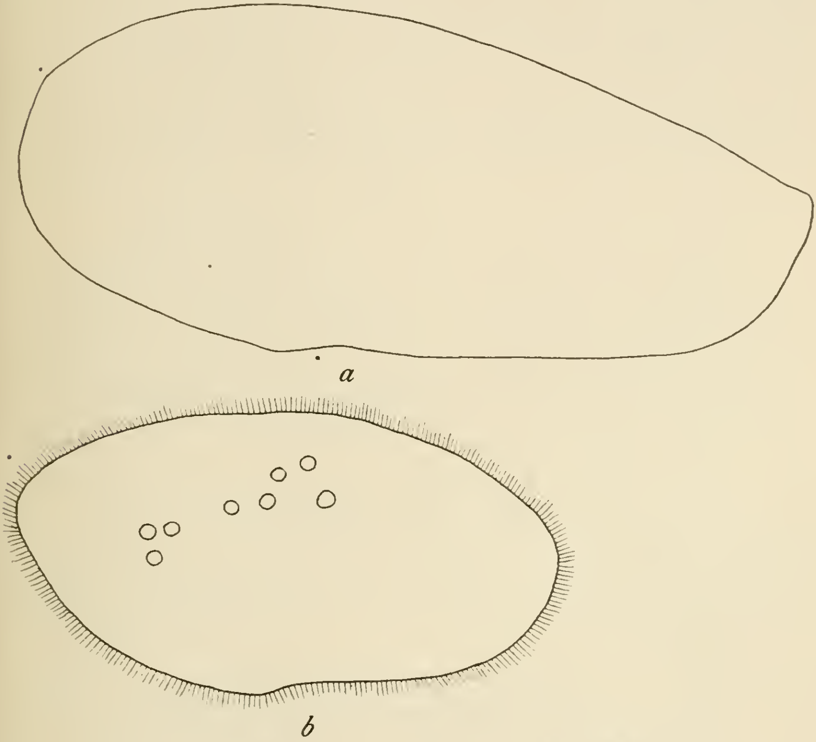


Fig. 111.—Cepedea buergeri sinensis, × 460 diameters.

CEPEDEA MINOR, new species.

Type.—United States National Museum Cat. No. 16583.

Host.—*Alytes obstetricans* (Laurenti), one scant but well preserved infection, from United States National Museum specimen No. 37194, 39 mm. long, from central France.

Measurements: A, of an average individual; B, of the largest individual seen—

Measurements.	A	B
	mm	mm
Length of body	0.097	0.137
Width of body	.0347	.046
Diameter of nucleus	.0037 to .0045	
Diameter of endospherule	.0015 and smaller	

This small *Cepedea* seems to be a distinct species, so far as one can judge from the material available. There is need of study of a series of infections from this host to determine if the impression from the few individuals now observed is confirmed after adequate data are gathered.

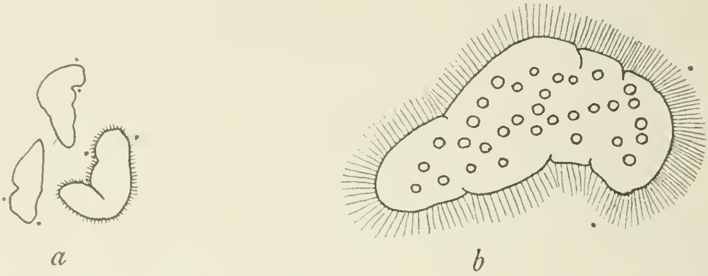


FIG. 112.—CEPEDEA MINOR: *a*, A GROUP OF CELLS, × 117 DIAMETERS; *b*, A CELL FROM THE SAME HOST, × 460 DIAMETERS, ALL THE NUCLEI BEING SHOWN.

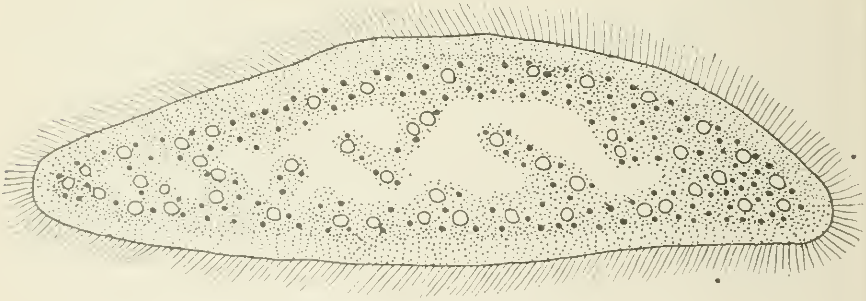


FIG. 113.—CEPEDEA PHRYNOMANTIDIS, × 460 DIAMETERS.

CEPEDEA PHRYNOMANTIDIS, new species.

Type.—United States National Museum Cat. No. 16513.

Host.—*Phrynomantis bifasciata* (Smith), one abundant infection, from United States National Museum specimen No. 20115, 28 mm. long, from Tana, southeast Africa, November, 1892; W. A. Chanler, collector.

Measurements of an ordinary individual.—Length of body 0.232 mm.; width of body 0.066 mm.; thickness of body 0.056 mm.; diameter of nucleus 0.0035 mm. to 0.0043 mm.; diameter of endospherule 0.001 mm. to 0.00132 mm.; cilia line interval, anterior 0.00132 mm., middle 0.003 mm., posterior 0.00475 mm.

This spindle-shaped *Cepedea* is somewhat *dimidiata*-like in form, but it differs from the *dididiata* group in having a greatly developed axial excretory vacuole. The much greater development of this structure in this species and in the species *madagascariensis*, *magna*, *obovoidea*, *globosa*, and *baudinii*, next to be described, seems to set them apart from the *dimidiata* group, on the one hand, and from the *longa* group, on the other hand.

CEPEDEA MADAGASCARIENSIS, new species (figs. 114 and 115).

Type.—United States National Museum Cat. No. 16514.

Host.—*Megalixalus madagascariensis* Duméril and Bibron, one infection, from United States National Museum specimen No. 33879, 26 mm. long, no date, no locality; F. Werner, collector.

Measurements: A, of a medium-sized individual; B, of a large individual—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.317	0.513
Width of body	.085	.07
Thickness of body:		
Anterior	.015	....
Middle	.019	....
Posterior	.0215	....
Length of nucleus	.0048	.0055
Width of nucleus	.0036	.003
Length of endospherule	.0011	.0019
Width of endospherule	.0011	.0012
Cilia line interval:		
Anterior	.0015	.002
Middle	....	.003
Posterior	.0027	.0036

The shorter individuals of this species are often considerably flattened, more so in front than behind. Others are very much elongated and more slender. The species seems to form a transition from the *dimidiata*-like forms to the *longa*-like forms on the one hand, and to the Opalinas, on the other hand. This interpretation is confirmed by study of two other species parasitic in two of the Bufos, *Cepedea magna*, a very slightly flattened form, and *C. obovoidea*, whose thickness is two-thirds of its width.

Figure 114 shows the posterior, excretory pore, and portions of the axial system of vacuoles. One sees that the endospherules are much more numerous in the anterior end of the body, as is usually the case, especially in the cylindrical genera *Cepedea* and *Protoopalina*. The nuclei are spherical and of moderate size.

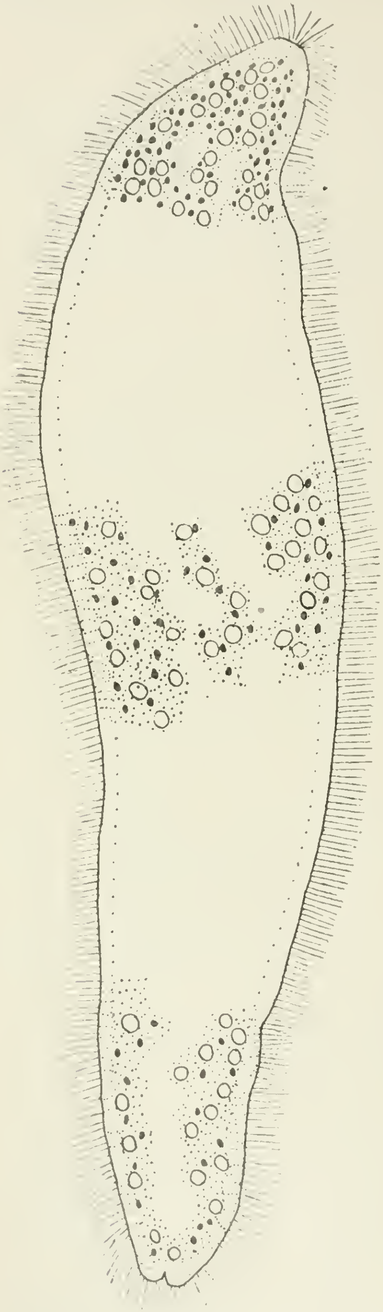


FIG. 114.—CEPEDEA MADAGASCARIENSIS, × 460 DIAMETERS.

CEPEDEA MADAGASCARIENSIS [of HYPEROLIUS]. Probably not a distinct subspecies (fig. 116).

A specimen of this form has been deposited with the United States National Museum as Cat. No. 16532.

Host.—*Hyperolius marmoratus* Rapp, one infection, from United States National Museum specimen No. 16053, 32 mm. long, from West Africa; W. H. Brown, collector.

Measurements of a good-sized individual.—Length of body 0.555 mm.; width of body 0.06 mm.; length of nucleus 0.0058 mm.; width of nucleus 0.0048 mm.; length of nucleus in mitosis 0.006 mm.; width of nucleus in mitosis 0.00375 mm.; diameter of endospherule 0.002 mm.

This form seems identical with *Cepedea madagascariensis* from *Megalixalus madagascariensis*, though its host and geographical locality are quite different. It approaches closely to *Cepedea longa* in the form of its body.

CEPEDEA MAGNA, new species (fig. 117).

Type.—United States National Museum Cat. No. 16515.

Host.—*Bufo latifrons* Boulenger, one abundant infection, from United States National Museum specimen No. 48855, 72 mm. long, from the Cameroons, West Africa; T. Barbour, collector.

Measurements of a good-sized individual.—Length of body 0.58 mm.; width of body 0.1045 mm.; thickness of body 0.085 mm.; length of nucleus 0.0035 mm.; width of nucleus 0.0025 mm.; diameter of endospherule 0.00125 mm.; cilia line interval, anterior 0.0025 mm., middle 0.0042 mm., posterior 0.0046 mm.

This large *Cepedea* has the *dimidiata* form and general character. It is slightly flattened. The well-developed axial series of vacuoles connects it clearly with the preceding two species.

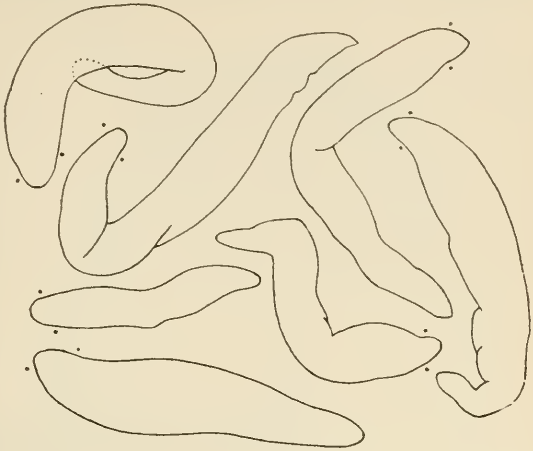


FIG. 115.—CEPEDEA MADAGASCARIENSIS, × 117 DIAMETERS.

CEPEDEA OBOVOIDEA, new species (fig. 118).

Type.—United States National Museum Cat. No. 16516.

Host.—*Bufo lentiginosus* Shaw, two infections, from Auburndale, Florida, March, 1912, N. R. Wood, collector. The type infection is from United States National Museum specimen No. 48779, 63 mm. long.

Measurements of a good-sized individual.—Length of body 0.315 mm.; width of body 0.0975 mm.; thickness of body 0.0653 mm.; length and breadth of nuclei, first specimen 0.0045 mm. by 0.0036 mm., second specimen 0.0045 mm. by 0.004 mm., third specimen 0.0054 mm. by 0.0047 mm.; diameter of endospherule 0.00125 mm.; cilia line interval, anterior 0.00117 mm., middle 0.0026 mm., posterior 0.004 mm.

This good-sized *Cepedea* is well demarcated from other species, 1, by its broad, rounded, anterior end; 2, by its long and very abundant cilia; 3, by the considerable flattening of its body. Its nuclei are broadly ellipsoidal. Its endospherules are small and globular.

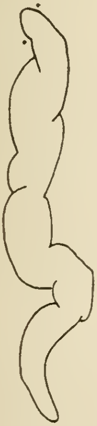


FIG. 116.—CEPEDEA MADAGASCARIENSIS [OF HYPEROLIUS], × 117 DIAMETERS.

In progressive flattening of the body, *Cepedea magna*, *C. obovoidea*, and *C. madagascariensis* form a series in the order named. There is no indication that the flattened forms (the genus *Opalina*) arose through these particular species, but these species show the probable manner of origin of the *Opalinae* by a progressive flattening of the body. Apparently this flattening began with the anterior end of the body, even so thick a form as *Cepedea dimidiata* form *zelleri* having its anterior end wedge-shaped.

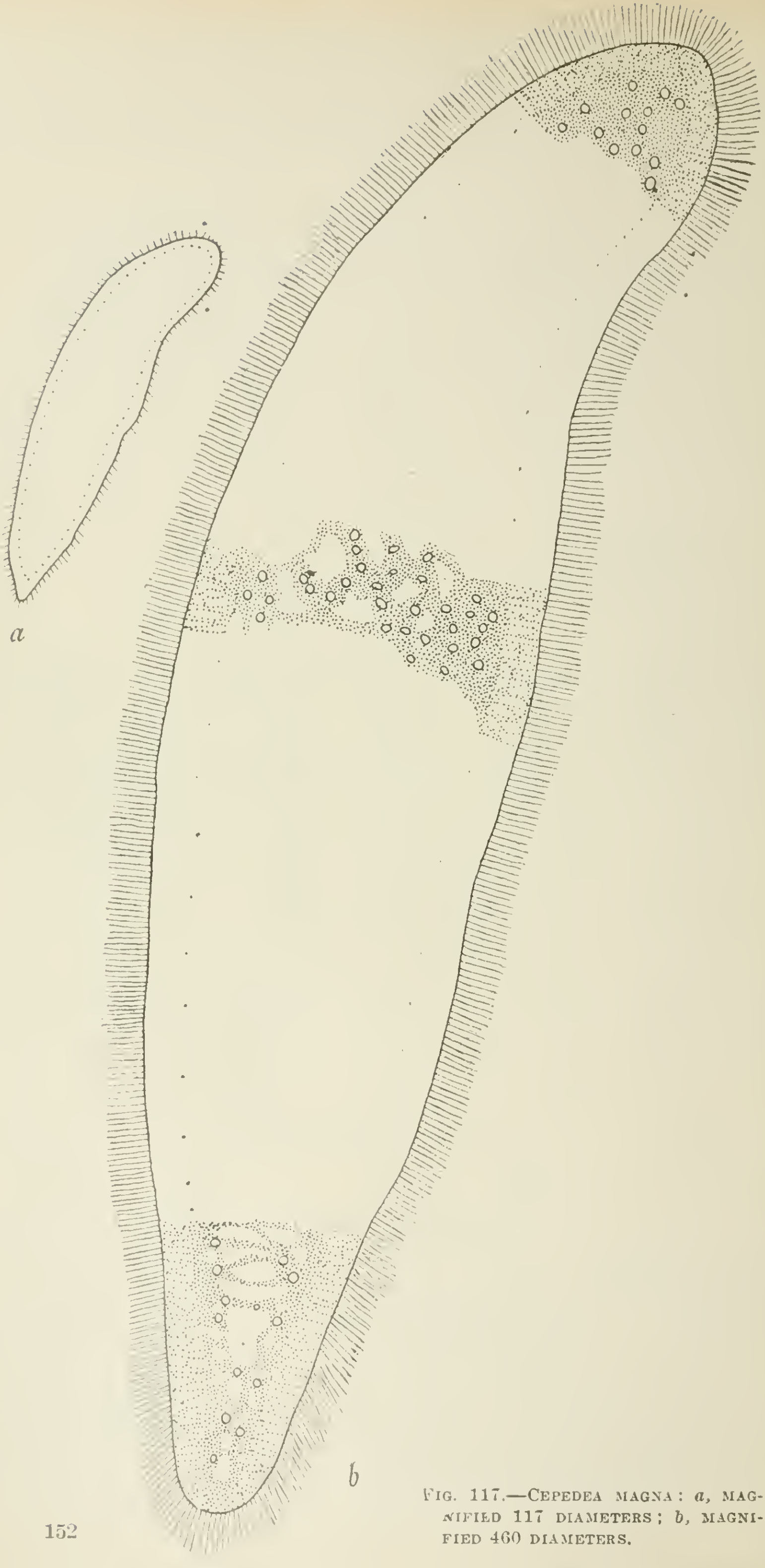


Fig. 117.—Cepedea magna: *a*, magnified 117 diameters; *b*, magnified 460 diameters.

CEPEDEA GLOBOSA, new species (fig. 119).

Type.—United States National Museum Cat. No. 16524.

Host.—*Phyllomedusa lemur* Boulenger, one good infection, from United States National Museum specimen No. 29935, 22 mm. long, from Turialba. Costa Rica: Burgdorf and Schild, collectors.

Measurements of an ordinary individual.—Length of body 0.17 mm.; width of body 0.113 mm.; thickness of body 0.656 mm.; length of nucleus 0.0065 mm.; width of nucleus 0.0045 mm.; length and breadth of endospherules, first 0.0022 mm. by 0.0022 mm., second 0.003 mm. by 0.0025 mm.: cilia line interval, anterior 0.002 mm., posterior 0.0046 mm.

This is a remarkably chunky *Cepedea*, densely clothed with long cilia. In my specimens the axial region of the body is greatly vacuolated, the irregular vacuole and its branches occupying more than half the thickness of the body. The body is considerably flattened, its thickness being about two-thirds its breadth. When discussing the *Opalinae* we will compare this species with a series of others of similar appearance, but increasingly flattened, namely, *O. helenae*, *O. helenae phyllomedusae*, and *O. morelletei*. These forms, parasitic in the Hylidae, in histological appearance and in the condition of the excretory vacuole resemble the forms *Cepedea phrynomantidis*.

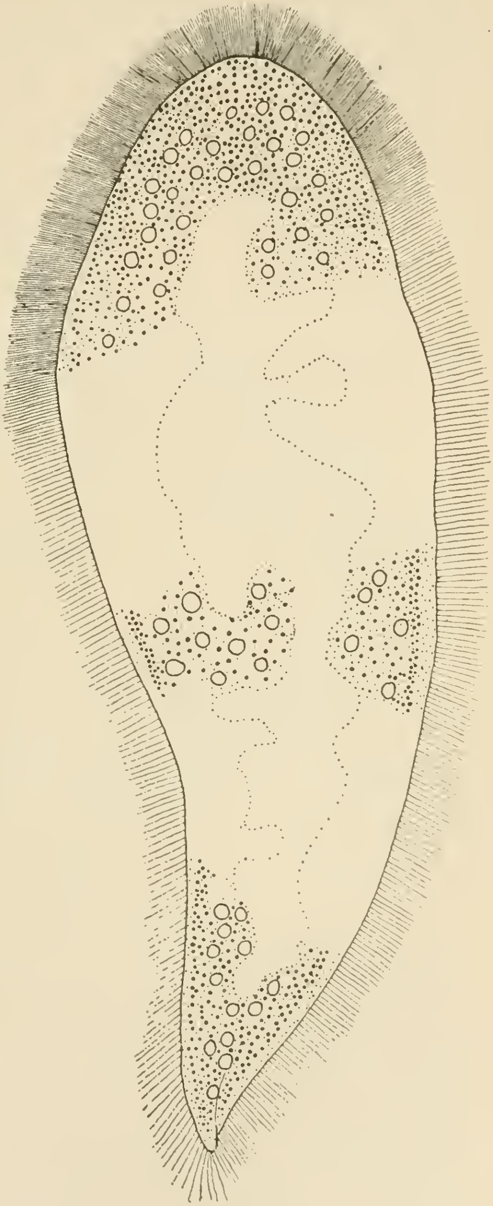


FIG. 118.—CEPEDEA OBOVOIDEA, × 460 DIAMETERS.

C. madagascariensis, *C. magna*, and *C. obovidea*. This group is but one of several series of intergrading forms between those Cepedeas which are more or less circular in cross section, and the much flattened Opalinas. The existence of several such series of intergrades seems to indicate the possibility of a polyphyletic origin for the forms placed in the genus *Opalina*.

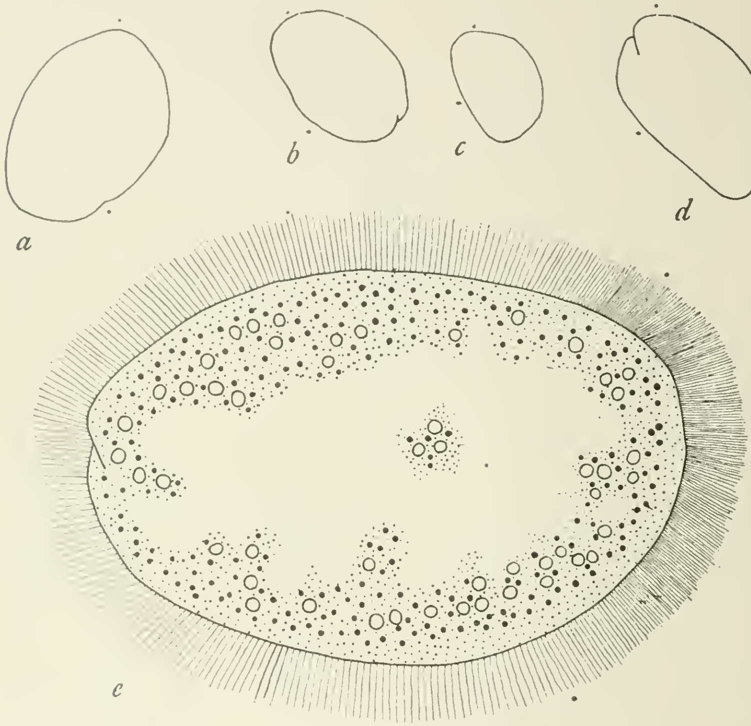


FIG. 119.—CEPEDEA GLOBOSA: *a* TO *d*, MAGNIFIED 117 DIAMETERS; *e*, MAGNIFIED 460 DIAMETERS.

CEPEDEA BAUDINII, new species.

Type.—United States National Museum Cat. No. 16525.

Host.—*Hyla baudinii* Duméril and Bibron, two infections, one from Guatemala and one, the type infection, from United States National Museum specimen No. 30410, 53 mm. long, from Cordova, Mexico; F. Sumichrast, collector.

Measurements of an ordinary individual.—Length of body 0.087 mm.; width of body 0.0475 mm.; thickness of body 0.0386 mm.; diameter of nucleus 0.00325 mm. to 0.00375 mm.; diameter of endospherule 0.0016 mm.; cilia line interval, anterior 0.00185 mm., middle 0.0025 mm., posterior 0.0025 mm.

This small, very stocky *Cepedea* somewhat resembles *C. globosa.* Its body is relatively thicker. It has the axial excretory vacuoles almost as much developed. Its nuclei, however, are spherical instead of ellipsoidal, and are much smaller.

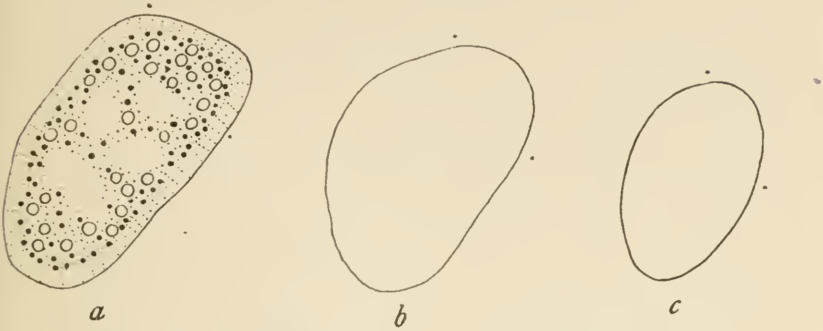


Fig. 120.—Cepedea baudinii, × 460 diameters.

CEPEDEA PULCHRA, new species.

Type.—United States National Museum Cat. No. 16504.

Host.—*Kaloula pulchra Gray*, one abundant infection, from United States National Museum specimen No. 10967, 51 mm. long, from Cochin China; donor, Museum of Natural History, Paris.

Measurements of a good-sized individual.—Length of body 0.142 mm.; width of body 0.0435 mm.; thickness of body 0.022 mm.; diameter of resting nucleus 0.0025 mm.; length and width of dividing nucleus, first nucleus 0.0034 mm. by 0.0026 mm., second nucleus 0.0035 mm. by 0.0029 mm., third nucleus 0.0045 mm. by 0.003 mm.; diameter of endospherule 0.001 mm.

This small *Cepedea* is considerably flattened and, like *C. madagascariensis*, represents probably an approach to the condition of the *Opalinae.* Its very small resting nuclei are spherical, but many nuclei are found in mitosis, the late anaphase being long and slender. These slender dividing nuclei, as well as the very small resting nuclei, readily distinguish this species from *C. dimidiata* and its several subspecies and from *C. madagascariensis.* Many individuals show a decided crowding of the endospherules in the anterior region of the body.

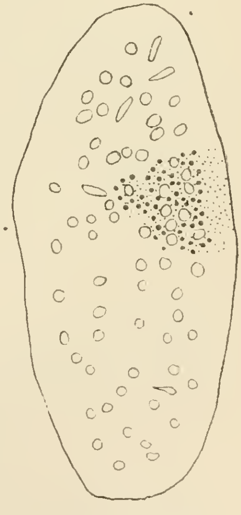


Fig. 121.—Cepedea pulchra, × 460 diameters.

CEPEDEA PULCHRA JAPONICA, new subspecies (fig. 122).

Type.—United States National Museum Cat. No. 16503.

Host.—*Rana rugosa* Schlegel, three infections, from Japan; the type infection is from United States National Museum specimen No. 31802, 33 mm. long, from Nara, Yamoto Province; H. M. Smith. collector.

Measurements of two individuals—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.11	0.1
Width of body	.035	.043
Thickness of body	.022	.029
Diameter of resting nucleus	.0035–.004	....
Length of dividing nucleus	.013	....
Width of dividing nucleus	.0035	....
Diameter of endospherule	.001	....

This is very similar to the species proper, except for its larger nuclei. Its cilia, better preserved than in the specimens of the species proper, are seen to be of considerable length.

CEPEDEA PULCHRA JAVENSIS, new subspecies (fig. 123).

Type.—United States National Museum Cat. No. 16623.

Host.—*Bufo melanostictus* Schneider, United States National Museum No. 43944, from Buitenzorg, Java, March 20, 1909; Bryant Javan Expedition, collectors.

Measurements: A of a rather small individual; B of a large individual—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.093	0.115
Width of body	.039	.053
Diameter of resting nucleus	.00375	.00375
Length of dividing nucleus	.0044	.0044
Width of dividing nucleus	.003	.003

This Javan *Cepedea* closely resembles *C. pulchra japonica*, found in *Rana rugosa*. The usual form and the range of variety of form are similar, but there is one marked difference. In the Japanese specimens the numerous nuclei in mitosis are greatly elongated, while in the Javan specimens the very few dividing nuclei are spindle-shaped and very short, not half again as long as broad. The frequency of dividing nuclei in the material may depend upon the time of year or the method of preservation or the conditions under which the specimens were kept prior to preservation, but the form of the mitotic nucleus is constant. Similar differences between species in the shape of the dividing nuclei have been observed in numerous instances and the character is found to be of diagnostic value. It hardly seems justifiable, however, on the basis of this character alone, to recognize the Javan forms as belonging to a distinct species. They are therefore placed as a subspecies of *Cepedea pulchra.*

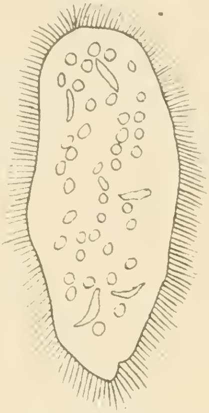


FIG. 122.—CEPEDEA PULCHRA JAPONICA, × 460 DIAMETERS.

CEPEDEA OCCIDENTALIS, new species (fig. 124).

Type.—United States National Museum Cat. No. 16505.

Host.—*Rana chrysoprasina* (Cope), one infection, in United States National Museum, specimen No. 14180, 23 mm. long, from Nicaragua, August 7, 1885; J. F. Bransford, collector.

Measurements of an ordinary individual.—Length of body 0.15 mm.; width of body 0.083 mm.; thickness of body 0.038 mm.; diameter of resting nucleus 0.004 mm. to 0.0045 mm.; diameter of endospherule 0.00175 mm.

This is another considerably flattened form, rather similar to *C. pulchra.* Its nuclei are even larger than those in *C. pulchra japonica.* Its endospherules are nearly twice as large as those of the latter variety. *Cepedea pulchra* and its subspecies and *C. occidentalis* seem to show a condition intermediate between that of the Cepedeas of the *dimidiata* group and that of the Opalinas.

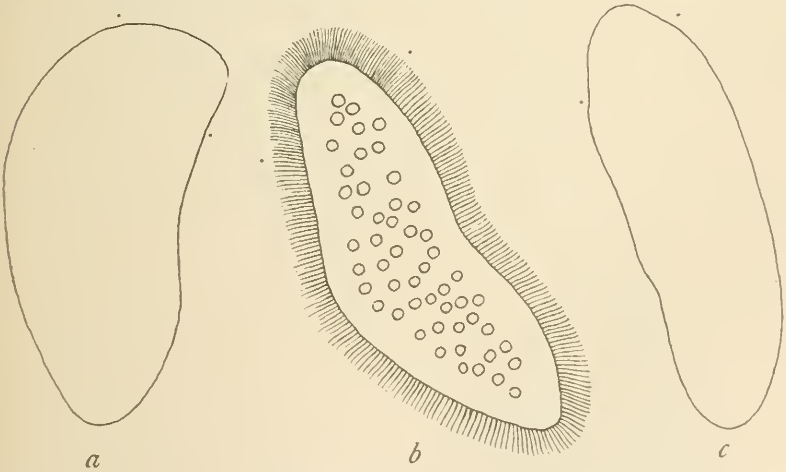


FIG. 123.—CEPEDEA PULCHRA JAVENSIS, × 460 DIAMETERS.

CEPEDEA FLORIDENSIS, new species.

Type.—United States National Museum Cat. No. 16518.

Host.—*Scaphiopus albus* Garman, one abundant infection, in United States National Museum specimen No. 52403, 54 mm. long, from Key West, Florida; A. Garman, collector.

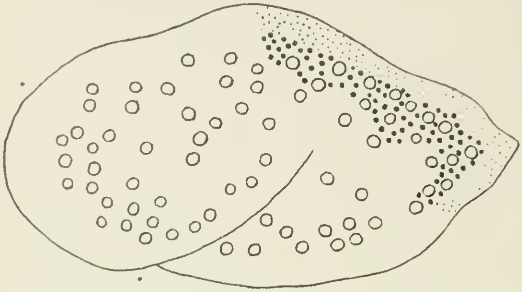


Fig. 124.—Cepedea occidentalis, × 460 diameters.

Measurements of an ordinary individual.—Length of body 0.23 mm.; width of body 0.089 mm.; thickness of body, anterior 0.018 mm. (width at same level 0.048 mm.), middle 0.025 mm. (width at same level 0.089 mm.), posterior 0.032 mm. (width at same level 0.048 mm.); diameter of resting nucleus 0.0036 mm. to 0.005 mm.; length and width of dividing nuclei, first nucleus 0.0047 mm. by 0.0027 mm., second nucleus 0.0048 mm. by 0.0034 mm., third nucleus 0.00525 mm.

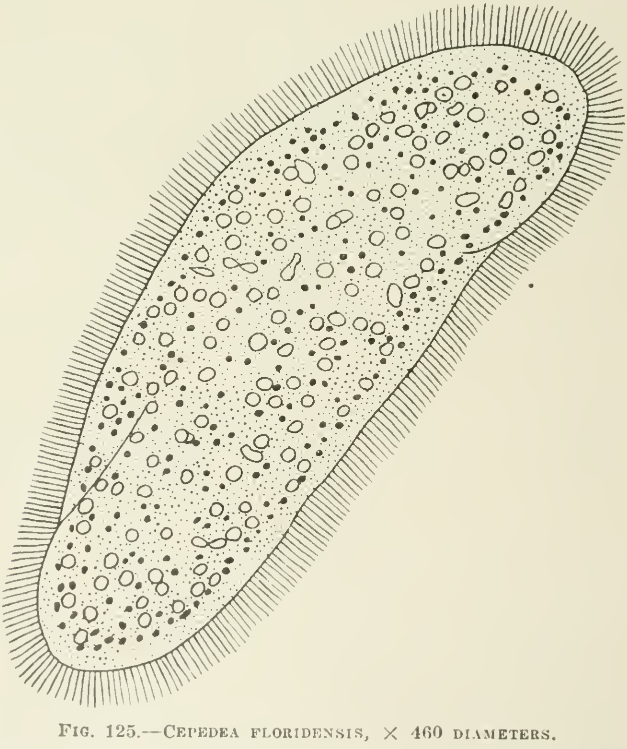


Fig. 125.—Cepedea floridensis, × 460 diameters.

by 0.004 mm., fourth nucleus 0.0055 mm. by 0.0032 mm.; length and width of endospherule, first 0.0013 mm. by 0.0009 mm., second 0.0019 mm. by 0.0011 mm.; cilia line interval, anterior 0.00166 mm., middle 0.00383 mm.

The general form of the body and the appearance of the nuclei of this *Cepedea*, in spite of its being very thin in front, suggests comparison with *C. occidentalis*, *C. pulchra*, and its subspecies *japonica*. In the posterior part of the body *C. floridensis* is nearly as thick as broad, but in front it is much thinner. The *Opalinae* have bodies of nearly uniform thickness throughout.

CEPEDEA BORNEONENSIS, new species.

Type.—United States National Museum Cat. No. 16517.

Host.—*Bufo jerboa* Boulenger, one good infection, from United States National Museum specimen No. 33880, 38 mm. long, from western Borneo; F. Sumichrast, collector.

Dimensions of an average individual.—Length of body 0.0525 mm.; width of body 0.0153 mm.; length of nucleus 0.004 mm.; width of nucleus 0.0022 mm.

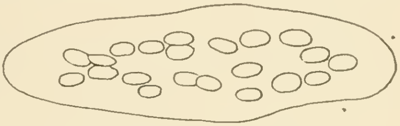


FIG. 126.—CEPEDEA BORNEONENSIS, × 1,000 DIAMETERS.

This very small *Cepedea* is spindle-shaped, with both ends rounded. Its ellipsoidal nuclei are large compared with the size of the body and they lie with their long axes parallel, or nearly parallel, to the long axis of the body. Its cilia seem to be very short, but it may be they are not well enough preserved to show their normal length. This seems a well demarcated species.

CEPEDEA FUJIENSIS, new species.

Type.—United States National Museum Cat. No. 16522.

Host.—*Bufo formosus* Boulenger, one very scant infection of well preserved Opalinids, from United States National Museum specimen No. 34324, 134 mm. long, from Fuji, Japan, August, 1898; A. Owston, collector.

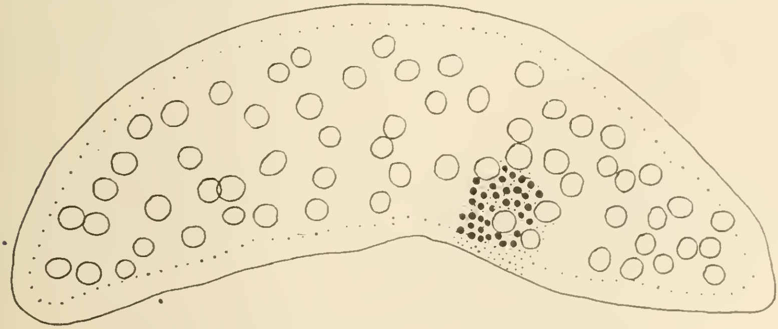


FIG. 127.—CEPEDEA FUJIENSIS, × 460 DIAMETERS.

Measurements.—Length of body 0.252 mm.; width of body 0.072 mm.; diameter of nucleus 0.00775 mm.; diameter of endospherule 0.00225 mm.; cilia line interval, anterior 0.00223 mm., middle 0.0036 mm., posterior 0.004 mm.

This is a good-sized *Cepedea* shaped like a rather stocky *C. dimidiata.* Its spherical nuclei are very large and its endospherules also are large and spheroidal. Although this is a scant infection, the few individuals found are of so distinct a sort that one does not hesitate to assign them to a distinct species.

CEPEDEA MEXICANA, new species.

Type.—United States National Museum Cat. No. 16502.

Host.—*Rana pipiens* Schreber, one infection, from United States National Museum specimen No. 3295, 72 mm. long, from Charco Escondino, Matamoros, Tamaulipas, Mexico, March, 1853; Couch, collector.

Measurements: A, of a large individual; B, of a small individual—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.217	0.1325
Width of body	.06	.0175
Length of nucleus	.0035 .0052	.004 .005
Width of nucleus	.003 .0027	.002 .0025
Cilia line interval:		
Anterior	.00187	....
Posterior	.0032	....

These Cepedeas, after 65 years in the rectum of the preserved frog, are in fair condition for study, though internal details of structure are not well seen. The body form is shown in the figure. The nuclei are distinctly ellipsoidal, in many cases being twice as long as broad. They lie with their long axes in general parallel, or nearly parallel with the major axis of the body. The cilia are short. The rather slender form of the body, and especially the very small ellipsoidal nuclei are distinguishing characters of this small *Cepedea.*

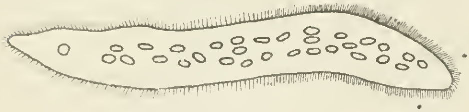


FIG. 128.—CEPEDEA MEXICANA, × 460 DIAMETERS.

CEPEDEA FORMOSAE, new species.

Type.—United States National Museum Cat. No. 16523.

Host.—*Bufo melanostictus* Schneider, three infections; one, scant, from United States National Museum specimen No. 37511, from

Hong Kong, China; and two others from Formosa, September, 1896; T. Tada, collector. Of the latter, United States National Museum specimen No. 36499 furnishes the type infection.

Measurements of an ordinary individual.—Length of body 0.17 mm.; width of body 0.02 mm.; length and width of nucleus, first nucleus 0.00435 mm. by 0.00435 mm., second nucleus 0.005 mm. by 0.00317 mm.; diameter of endospherule 0.0014 mm.; cilia line interval, anterior 0.0015 mm., middle 0.00375 mm., posterior 0.00375 mm.

This spindle-shaped *Cepedea* much resembles *C. dimidiata* in form. Some of its nuclei are spherical, more are ellipsoidal. Its cilia are rather long.

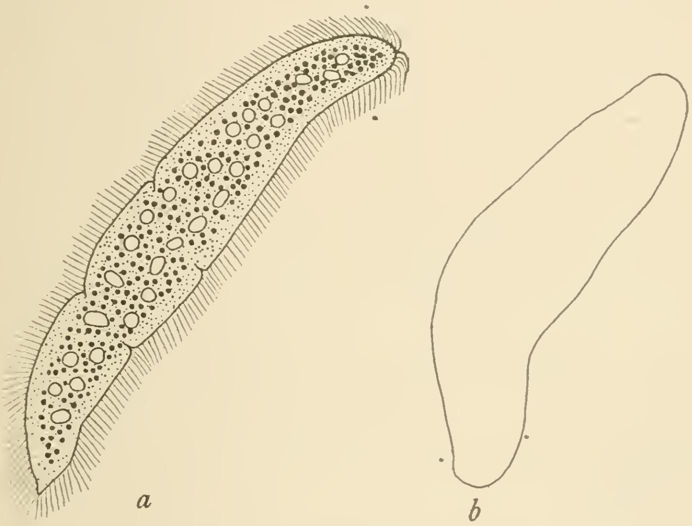


FIG. 129.—CEPEDEA FORMOSAE, × 460 DIAMETERS.

In a number of individuals one sees an interesting condition. There are often one or two or more transverse constructions of the body, divisions which have started but are not yet completed. This feature is more developed in *C. segmentata*.

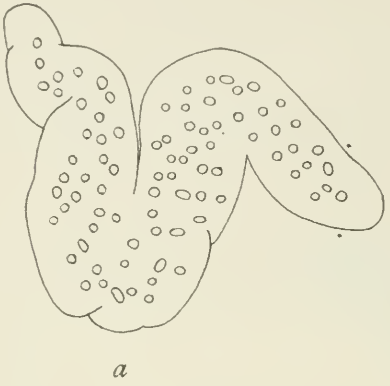
CEPEDEA HISPANICA, new species.

Type.—United States National Museum Cat. No. 16510.

Host.—*Rana esculenta hispanica* (Michahelles), three good infections, from Alicante Province, Spain, January 25, 1907; Thomas and Miller, collectors. Of these, the type infection is from United States National Museum specimen No. 38481, 48 mm. long.

Measurements.—Length of body 0.3 mm.; width of body 0.05 mm.; length and width of nucleus, first nucleus 0.0045 mm. by 0.0035 mm., second nucleus 0.0063 mm. by 0.0045 mm.; diameter of endospherule 0.001 mm.; cilia line interval, anterior 0.0012 mm., middle 0.0024 mm., posterior not clear.

This large *Cepedea*, though found in a subspecies of *Rana esculenta*, differs clearly from the *Cepedea* found in the latter host. It is a large, slender form with all its nuclei oval. It belongs more to the *longa* group than to the *dimidiata* group, though its short cilia and small endospherules are like those of *C. dimidiata*. It seems as intermediate species between the *dimidiata*-like forms and the very elongated forms. Many of the individuals in my infections are coiled and twisted, as I have never seen *C. dimidiata*, and as *C. longa* usually is found to be. Its oval nuclei also are *longa*-like.



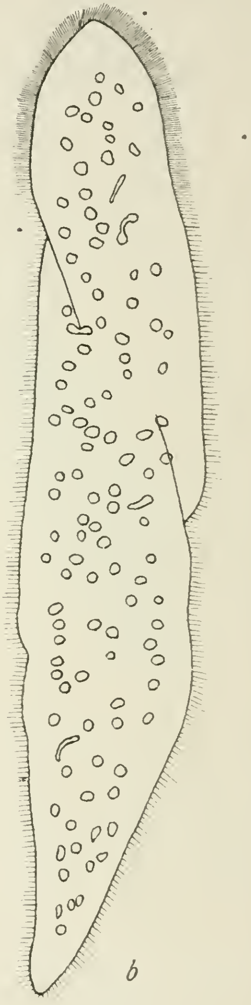


Fig. 130.—Cepedea hispanica, × 460 diameters.

CEPEDEA CANTABRIGENSIS, new species.

Type.—United States National Museum Cat. No. 16552.

Hosts.—*Rana cantabrigensis* Baird, five infections, from northwestern United States and Manitoba; and *Rana cantabrigensis latiremis* (Cope), five infections, from Alaska; of the latter the type infection is from United States National Museum specimen No. 15488, from Fort Cosmos, northern Alaska; Stoney, collector.

Measurements of a medium-sized individual.—Length of body 0.346 mm.; width of body 0.084 mm.; length and breadth of nuclei, first nucleus 0.005 mm. by 0.004 mm., second nucleus 0.00575 mm. by

0.004 mm., third nucleus 0.0055 mm. by 0.0055 mm.; cilia line interval, anterior 0.00235 mm., posterior 0.00325 mm.

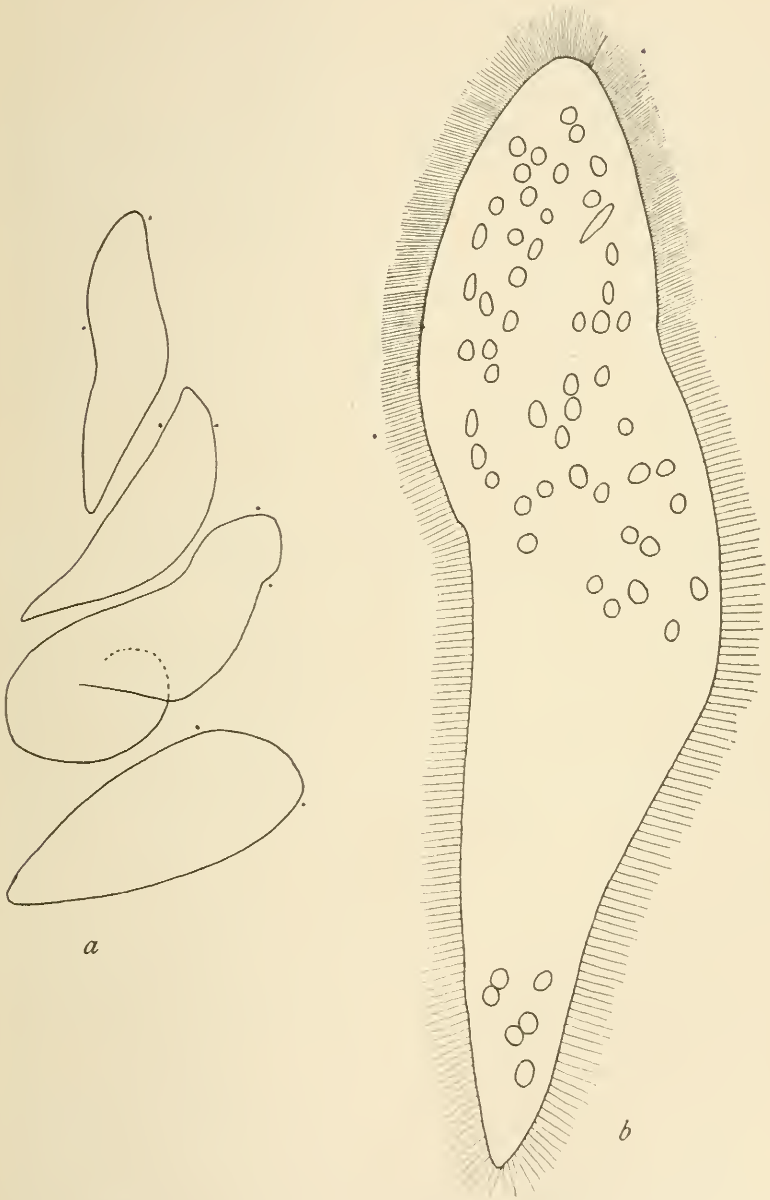


Fig. 131.—Cepedea cantabrigensis, from Rana cantabrigensis latiremis: *a*, a group of cells magnified 117 diameters; *b*, magnified 460 diameters.

This *Cepedea* has long cilia. Its nuclei are mostly ellipsoidal, though not much elongated except in active mitosis. It suggests com-

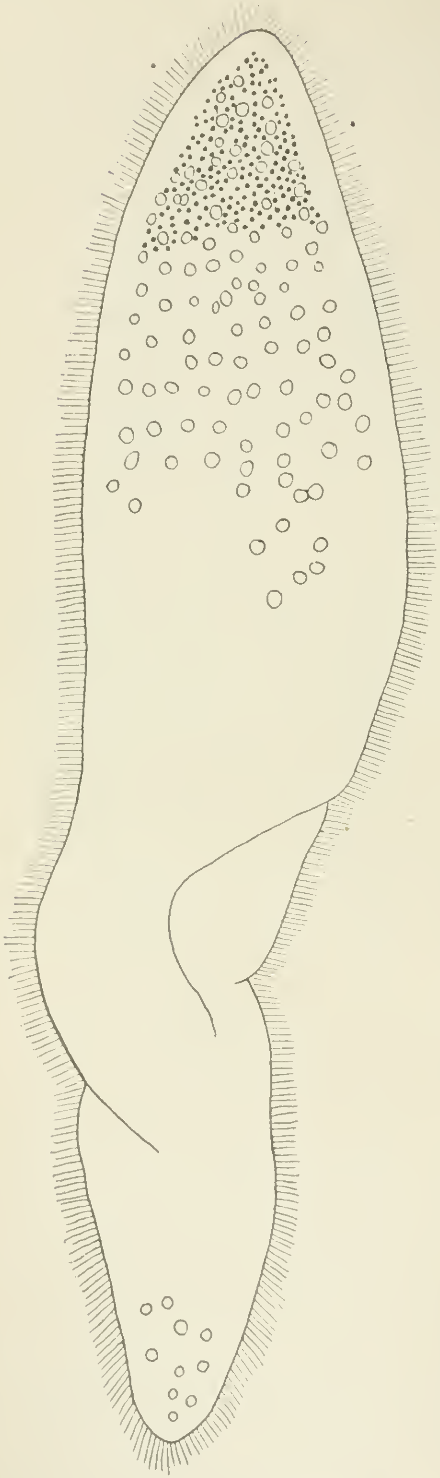


FIG. 132.—CEPEDEA CANTABRIGENSIS, FROM RANA CANTABRIGENSIS, × 460 DIAMETERS.

parison with the considerably flattened *C. madagascariensis*, but has, in general, a broader body form and larger nuclei, mostly ellipsoidal instead of spherical.

Cepedea cantabrigensis, United States National Museum Cat. No. 16553 (Fig. 132).

Longer forms with shorter cilia and slightly smaller nuclei are seen in *Rana cantabrigensis*, United States National Museum specimen No. 3975, 38 mm. long, from Rapid River, Minnesota, August 9, 1894; A. J. Woolman, collector.

Measurements of a good-sized individual.—Length of body 0.41 mm.; width of body 0.0946 mm.; thickness of body 0.0278 mm.

Cepedea cantabrigensis, United States National Museum Cat. No. 16554.

Still more elongated forms are seen in *Rana cantabrigensis latiremis*, United States National Museum specimen No. 13727, 51 mm. long, from Lake Alloknagik, Alaska, June, 1898; C. L. McKay, collector. These are very slender posteriorly, many individuals being sharp pointed.

CEPEDEA MULTIFORMIS, new species (fig. 133).

Type.—United States National Museum Cat. No. 16527.

Host.—*Hyla albomarginata* Spix, three infections, two from Nicaragua, and one, the type infection, from United States National Museum specimen No. 48856, 55 mm. long, a female with small ovary and immature eggs, from Bonito, Brazil; Branner, collector.

Measurements: A, of a small individual; B, of an elongated individual—

Measurements.		*A*	*B*
		mm.	*mm.*
Length of body		0.152	0.2174
Width of body		.0413	.05
Thickness of body, anterior		.013	....
Width at same level,		.0456	
Thickness of body, middle		.033	.048
Width at same level,		.037	
Thickness of body, posterior		.021	....
Width at same level,		.021	
Length of nucleus	No. 1	.004	.0052
	No. 2	.0035	.0058
Width of nucleus	No. 1	.0033	.0038
	No. 2	.0028	.004
Length of endospherule		.0017	.001
Width of endospherule		.001	.001
Cilia line interval:			
Anterior		.00175	.0014
Middle		....	.0017
Posterior		.00275	.0032

The shorter and intermediate forms of this *Cepedea* quite closely resemble *C. hispanica*, but the more elongated forms approach *C. longa*, or better, *C. ophis* in shape. This species evidently shows a nearer approach to the very elongated species than does *C. hispanica.* The cilia are quite long, longer than in *C. dimidiata* and its several subspecies, longer than in *C. longa*, but not so long as in *C. ophis* and *C. segmentata.* If these species were arranged in natural series they would be in the order *C. dimidiata*, *C. hispanica*, *C. multiformis*, *C. ophis*, *C. longa.* We will, however, take up later the latter two forms in reverse order without implication as to phylogeny.

CEPEDEA MULTIFORMIS [or POLYPEDATES SCHLEGELII], new subspecies (?) (fig. 134).

A specimen of this form has been deposited with the United States National Museum as Cat. No. 16529.

Host.—Polypedates schlegelii Guenther, four infections, from Japan. The type infection is from United States National Museum specimen No. 23589, 48 mm. long, from Yokohama, September 1896; L. Stejneger collector.

Measurements.—Length of body 0.26 mm.; width of body 0.06 mm.; length and width of nucleus, first nucleus 0.0055 mm. by 0.0028 mm., second nucleus 0.0055 mm. by 0.0037 mm. (mean), third nucleus 0.0065 mm. by 0.00376 mm.; diameter of endospherule 0.0013 mm. to 0.0016 mm.; cilia line interval, anterior 0.0025 mm.

This form seems very similar to the species type, although it is

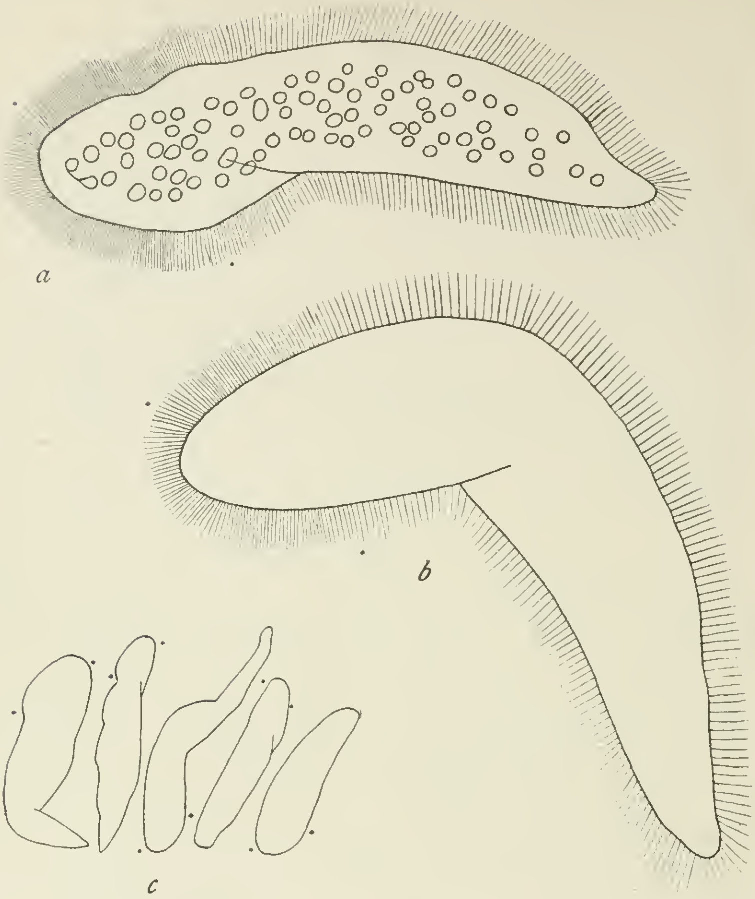


Fig. 133.—Cepedea multiformis: *a* and *b*, magnified 460 diameters; *c*, a group of cells magnified 117 diameters.

parasitic in one of the Ranidae from eastern Asia, instead of in a Hyla from Central America. It is only because of its very different host and because of its different geographical locality that it is not assigned without hesitation to the main species. The forms seem practically identical. The bracketed subspecific name is given for convenience of reference.

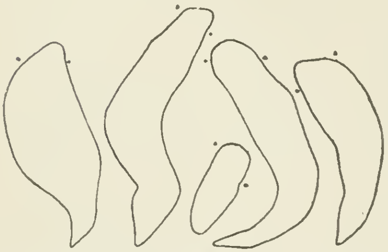


Fig. 134.—Cepedea multiformis [of Polypedates schlegelii], × 117 diameters.

CEPEDEA SEYCHELLENSIS, new species.

Type. — United States National Museum Cat. No. 16531.

Host.—*Megalixalus seychellensis* (Tschudi), two abundant infections, from Ile Mahé, Seychelles Islands, 1892; W. L. Abbott, collector. The type infection is from United States National Museum specimen No. 20415, 72 mm. long.

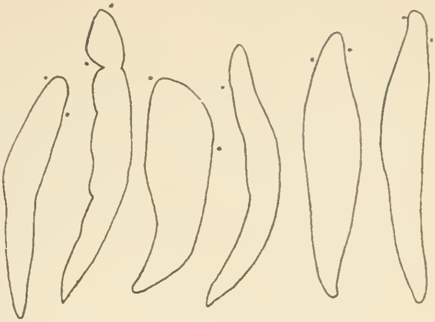


FIG. 135.—CEPEDEA SEYCHELLENSIS, × 117 DIAMETERS.

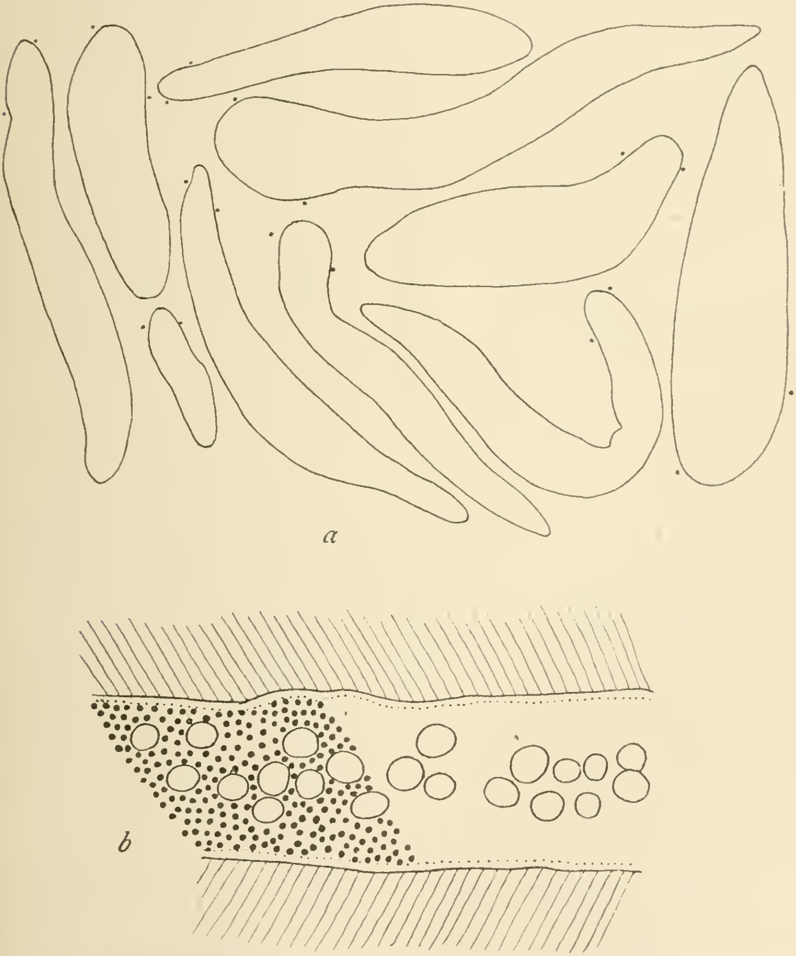


FIG. 136.—CEPEDEA DOLICHOSOMA: *a*, A GROUP OF ANIMALS MAGNIFIED 117 DIAMETERS; *b*, A SMALL PORTION OF THE BODY OF ONE INDIVIDUAL, IN OPTICAL SECTION, × 870 DIAMETERS.

Measurements of a good-sized individual.—Length of body 0.34 mm.; width of body, 0.051 mm.; diameter of nuclei 0.0026 mm.. 0.0035 (mean), some nuclei 0.0045 mm. by 0.0035 mm.; diameter of endospherule 0.0017 mm. (a few endospherules are 0.0017 mm. by 0.0008 mm. as if divided); cilia line interval, anterior 0.0022 mm., posterior 0.0034 mm.

This Cepeda is somewhat like *C. multiformis*, but is more spindle-shaped and has smaller nuclei which are spherical, or nearly so. Compare here *C. madagascariensis*, whose longer individuals show an approach to *C. longa*.

CEPEDEA DOLICHOSOMA, new species (fig. 136, p. 167).

Type.—United States National Museum Cat. No. 16530.

Host.—*Bufo haematiticus* Cope, five infections, one from Costa Rica and four from Nicaragua, August 7, 1885; J. F. Bransford, collector; all of the latter, including the type infection, are from a jar of United States National Museum specimens numbered 14181. The host furnishing the type infection is 38 mm. long.

Measurements of a large individual.—Length of body 0.848 mm.; width of body 0.03 mm.; diameter of average nucleus 0.0053 mm.

This is another multiform species. The longest, slenderest individuals, except for their spherical nuclei, would be assigned to the species *C. longa*, but other individuals much resemble some of the forms of *C. multiformis*. It seems to be an intermediate species. more *longa*-like than *C. multiformis*.

CEPEDEA LONGA (Bezzenberger).

Opalina longa BEZZENBERGER (1904).

A specimen of this form has been deposited with the United States National Museum as Cat. No. 16519.

Host.—*Rana limnocharis* Wiegmann, reported by Bezzenberger from "Asia." I have had three infections from Japan and one from Formosa. The example of this species which I have deposited with the United States National Museum as No. 16519 is from United States National Museum specimen of this frog No. 36502, 41 mm. long, from Giilan, Formosa, September, 1906; T. Tada, collector.

Measurements: A, after Bezzenberger; B, of an ordinary individual from one of my infections—

Measurements.	A	B
	mm.	*mm.*
Length of body	0. 68	1
Width of body	.052	.075
Dimensions of nucleus	0. 0053 × .0045 .0075 × .00525	0. 0055 × .0035 .0041 × .0032
Diameter of endospherule	.0025±	.0025
Cilia line interval, anterior	.00226	.0014
Length of basal granule of cilia	.004	Minute, spherical.
Width of same	.00033	

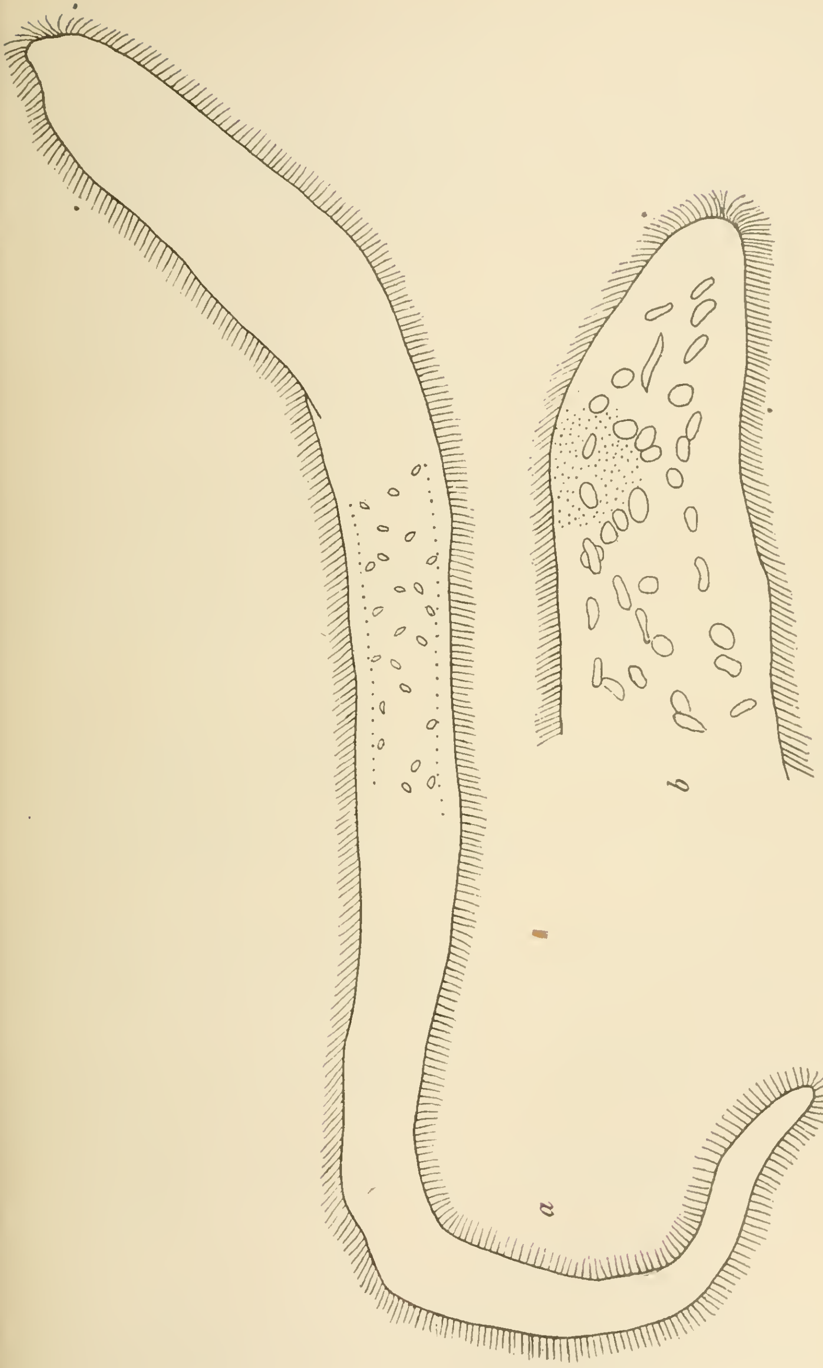


FIG. 137.—CEPEDEA LONGA: *a*, MAGNIFIED 249 DIAMETERS; *b*, ANTERIOR END OF ANOTHER INDIVIDUAL, × 1,000 DIAMETERS.

Bezzenberger (1904) gives a good description of this species with good figures. The body is greatly elongated, rounded in front and tapering posteriorly to a rounded point. The anterior end of the body is flattened so that in longitudinal section it would be wedge-shaped. In cross section the body is broadly elliptical. Bezzenberger says the nuclei are spherical or ellipsoidal, of a diameter of 4.5μ to 5.3μ, but one of his figures shows ellipsoidal nuclei 5.25μ *x* 7.5μ. He figures and describes the basal granules of the cilia as very slender, elongated rods 4μ by 0.33μ, a feature difficult to understand. There may be some confusion here. If not, then Bezzenberger's *Opalina* [*Cepedea*] *longa* differs most decidedly from my specimens of what seems to be this species, and from all other known Opalinids, in which, without exception, the basal granules of the cilia are spherical, or nearly so. Bezzenberger figures the cilia as of moderate length.

My specimens of the Opalinid from *Rana limnocharis* are larger than Bezzenberger's and have nuclei in general more elongated and smaller than those he figures, and as noted the basal granules of the cilia are very different. It seems there must be some error in the latter feature of Bezzenberger's description. The other divergencies between his specimens and mine it seems best to regard as racial rather than specific.

CEPEDEA, species? (fig. 138).

Host.—*Hyla veriscolor chrysoscelis* Cope, one very scant infection, from New Braunfels, Texas, from United States National Museum specimen No. 3234, 40 mm. long, collected by Lindheimer. One individual only was found, along with specimens of *Opalina*, species?. (See p. 221.)

Measurements of an average individual.—Length of body, 0.343 mm.; width of body, 0.0346 mm.; diameter of nucleus, 0.0034 mm. to 0.00425 mm.; diameter of endospherule, 0.0015 mm.; cilia line interval, anterior 0.0018 mm., posterior 0.00325 mm.

This *Cepedea* looks somewhat like a short *C. longa*. It has cilia of about the same length. The nuclei of this Opalinid are spherical, with the exception of a few in mitosis. The axial region of the endoplasm is less dense than its outer portion. Of course it is not well to name a new species from this single specimen. It has been deposited with the National Museum in a slide of *Opalina*, species (?) as Cat. No. 16579.

CEPEDEA OPHIS, new species (fig. 139, p. 172).

Type.—United States National Museum Cat. No. 16520.

Host.—*Rana tigerina* Daudin, two scant infections from Formosa, and two very abundant infections from Billeton Island, near Sumatra, August 8, 1904, W. L. Abbott collector; of these latter, the type infection is from United States National Museum specimen No. 35297.

Measurements.—Length of body, 0.5 mm.; width of body, 0.025 mm; diameter of nucleus, 0.0035 mm. to 000435 mm.; diameter of endospherule, 0.00125 mm. to 0.0015 mm.; cilia line interval, anterior 0.0018 mm., middle 0.0021 mm., posterior 0.0025 mm.

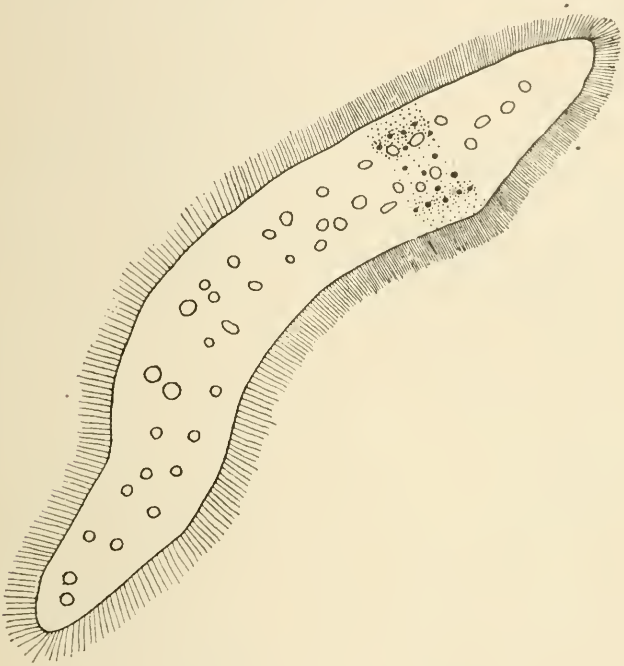


FIG. 138.—CEPEDEA, SPECIES?, × 460 DIAMETERS.

This *Cepedea* resembles *C. longa* in form of body, but has cilia at least twice as long, and has spherical instead of elongated nuclei. In one infection the individuals are shorter, as if they had been formed by transverse division. Chiefly because of the markedly longer cilia and the different shape of the nuclei, this must be regarded as a distinct species.

With this *Cepedea*, in the same infection are numerous specimens of *Protoopalina filiformis*.

CEPEDEA SEGMENTATA, new species (fig. 140, p. 173).

Type.—United States National Museum Cat. No. 16521.

Host.—*Polypedates leucomystax* Gravenhorst, one abundant infection from Cochin China, two scant infection from Buitenzorg, Java, and one infection, the type, from Sumatra, from United States

National Museum specimen No. 29437, 62 mm. long, a female with eggs; L. Karscher collector.

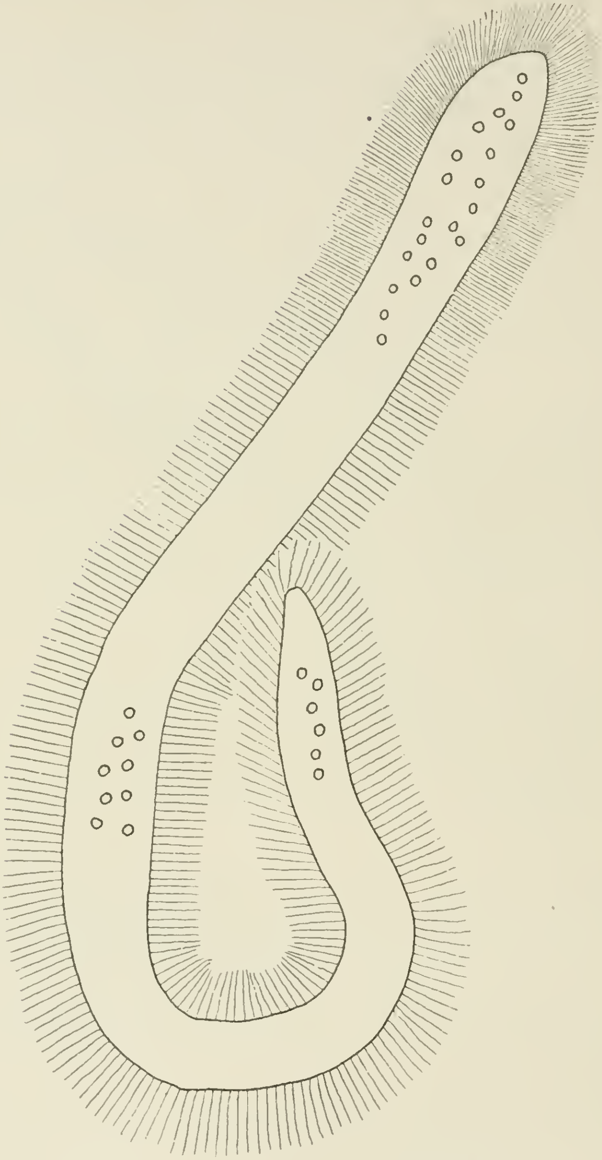


FIG. 139.—CEPEDEA OPHIS, × 460 DIAMETERS.

Measurements of a large individual.—Length of body, 0.365 mm.; width of body, 0.03 mm.; length and width of nuclei, first nucleus 0.0052 mm. by 0.0036 mm., second nucleus 0.0055 mm. by 0.0035 mm., third nucleus 0.005 mm. by 0.004 mm.; diameter of endospherule, 0.0015 mm.; cilia line interval, anterior 0.00112 mm.

My infections from *Polypedates leucomystax* show Opalinids of elongated, slender form, but shorter than typical *C. ophis*. They are perceptably more densely clothed with cilia. Their nuclei also are larger and are ellipsoidal, instead of spherical as in the latter species.

These animals show a remarkable series of transverse constrictions. It seems that many fission furrows have started without completely cutting through, so that the body appears as a series of segments. Some of these constrictions are deeper, some are shallower. It is evident that the first furrows have not been completed before new ones appear. In other Opalinids, previous to the period of sexual reproduction, we find a frenzy of fission, division furrows appearing so rapidly that a second or third division may be begun before the first is completed. The individual figured is not a very extreme example from its infection, but in two other infections no specimens are found with so many pseudometameres. Nearly all individuals, however, except the smallest, in all the infections studied show the "segmentation" more or less well developed. In section 5 of this paper the phenomena of speciation among the Opalinids will be treated in their broader aspects and this species will there be regarded as the last term in the evolution of the Opalinids so far as the tendency to inhibit and delay division of the body is concerned.

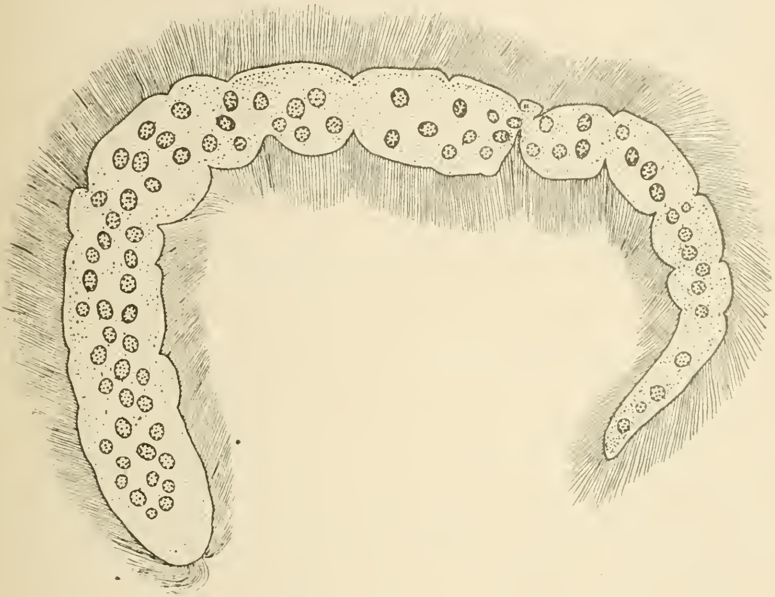


FIG. 140.—CEPEDEA SEGMENTATA, × 460 DIAMETERS. THE NUCLEI SHOULD BE SHOWN MORE ELONGATED.

CEPEDEA (?) FLAVA (Stokes).

Opalina flava STOKES (1884)

Host.—Scaphiopus solitarius Holbrook, one scant infection in a young individual, date and locality not mentioned, studied by Stokes (1884 and 1888).

Stokes' description is as follows.—

> *Opalina flava*, sp. nov. Body ovate, inflated, often as long as broad, or subpryriform, widest and rounded, posteriorly, the length one and one-half times the breadth; the right and left hand borders evenly rounded; striations of the cuticular surface obliquely disposed and bearing the long, fine, vibratile cilia; nuclei (?) numerous, small, scattered; sarcode enclosing many refractile corpuscles and larger spherical bodies apparently vacuolar; contractile vesicle none; parenchyma lemon yellow, the color darkest near the periphery, where it is disposed in a layer, the central portion of the sarcode being comparatively colorless. Length $\frac{1}{330}$ to $\frac{1}{350}$ of an inch. Habitat, the rectum of the spade-foot hermit toad, *Scaphiopus holbrooki* [*S. solitarius* Holbrook].

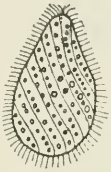


FIG. 141. — CEPEDEA (?) FLAVA. (AFTER STOKES.)

Stokes further says:

> The infusorian is broadly ovate, soft and flexible and somewhat changable in shape, assuming at will a subpyriform or subglobular figure.

Also:

> Its numbers are not great: perhaps a dozen were noted in the contents of the rectum. Neither is it always to be found.

Also:

> * * * associated with them was * * * a large species of *Opalina*, which I have, after some hesitation, identified with *O. ranarum* Purk. * * * Their appearance and structure are those of the latter, but the size is much less. They are quite active. As they pressed each other beneath the surface or forced each other upwards, the aspect of the field of view was comically like a pool of furiously boiling soup with big dumplings bobbing about.

It is difficult to determine what Opalinids Stokes saw in this infection. The yellow color emphasized in the name is found in the ectosarc of numerous species, perhaps of all under certain conditions (see Metcalf, 1909). The shape is not adequately described, the word " inflated " being insufficient to let us know whether the bodies were at all flattened, or were even considerably flattened but thick in comparison with the *ranarum*-like forms with which they were associated. " Subpyriform " and " subglobose " also are not clear as to the thickness of the animals. Such change of shape as Stokes describes I have not observed in any species, except when an individual is pressed out of shape by some external influence. Influenced by Stokes' use of the words " inflated," " subpyriform " and " subglobose," I am placing this species as one of the Cepedeas, and am not attempting to determine to what species it is most nearly related. Stokes' description does not fit any form I have studied. The species is of very doubtful validity.

The *ranarum*-like forms Stokes describes, of course, can not be identified. The species I have found in the same host is *Opalina obtrigonoidea.*

Genus OPALINA.

The uniformly and much flattened multinucleated forms have now been reached. Among them we can distinguish two general sorts, the *obtrigona*-like species, *Opalinae angustae*, in general oblanceolate in form, and the *ranarum*-like species, *Opalinae latae*, more rounded. Between the marked extremes of these two sorts are numerous intermediate forms, so that the two subgeneric groups are not sharply demarcated from each other. It seems possible that these much flattened, uniformly flattened species, the Opalinas, may have arisen independently several times from cylindrical forms. Indeed we have two, perhaps three, series of intergrades that suggest this. A number of the Cepedeas are quite flat anteriorly, and some species are also somewhat flattened even posteriorly. A multiple origin for the much flattened multinucleate forms seems the more possible, since among the binucleated Opalinids there are, on the one hand, the cylindrical or slightly flattened Protoöpalinas and, on the other hand, the much flattened Zelleriellas. This tendency toward flattening almost surely appeared at least twice in the family, once among the binucleated forms, and once among the multinucleated forms. The Zelleriellas may likely be monophyletic; at least we have no special indication of multiple origin. The flattening of Protoöpalinas to produce Zelleriellas seems a phenomenon parallel to the flattening of Cepedeas to produce Opalinas, and seemingly the latter process may have taken place more than once in the phyletic history. If this be true, the term *Opalina* used as a generic name for the much flattened species is not strictly a proper taxonomic term indicating true genetic relationship, but is rather a convenient designation for species of similar shape but probably somewhat diverse origin. We have not, however, the data for determining the genesis of the several species, so must retain the genus *Opalina* in this somewhat unscientific use.

Among the *obtrigona*-like *Opalinæ* there is such intergradation between species that here again, as among the Zelleriellas and Cepedeas, we can not always sharply and with certainty demarcate species, and the *ranarum*-like species present similar difficulties. Any classification must be somewhat arbitrary and conventional.

OPALINAE ANGUSTAE.

OPALINA OBTRIGONA Stein (1864).

Specimens of this species have been deposited with the United States National Museum as follows: Nos. 16533 and 16644 from

Hyla arborea, 16534 from *Hyla arborea japonica*, 16535 from *Hyla arborea savignyi.*

Hosts.—Hyla arborea (Linnaeus), reported by many observers; my material includes many infections studied in Germany: *Hyla arborea japonica* Schlegel, one infection from Japan, in United States National Museum specimen No. 23907, 32 mm. long, I. Ijima collector, and one infection from United States National Museum specimen No. 52354, 35 mm. long, from Yulu River, southern Manchuria, A. de C. Sowerby collector (but one small clump of Opalinae was seen in this last infection and they were not studied enough to make the identification certain); *Hyla arborea savignyi* Audouín, one infection in United States National Museum specimen No. 37190, 40 mm. long, from Jerusalem, Werner collector.

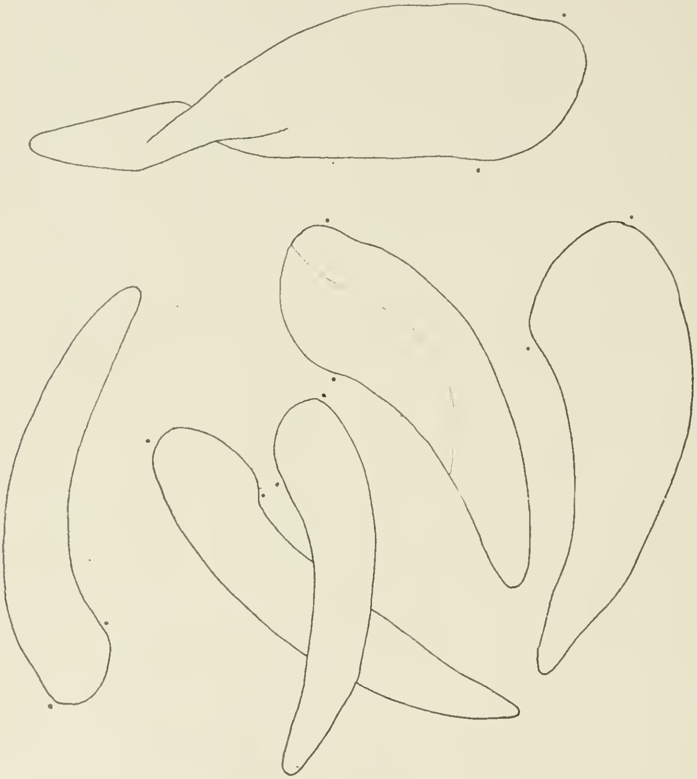


Fig. 142.—Opalina obtrigona, × 117 diameters. In one figure is drawn a single line of insertion of a row of cilia to indicate its spiral course.

Measurements of an average individual from Hyla arborea.—Length of body 0.483 mm.; width of body 0.154 mm.; thickness of body, anterior 0.034 mm., middle 0.0345 mm., posterior 0.037 mm.; diameter of nucleus 0.00675 mm. to 0.009 mm.; diameter of endospherule 0.0015 mm. (some were elongated, 0.0015 mm. by 0.0025;

mm.) cilia line interval, anterior 0.0025 mm., middle 0.00475 mm., posterior 0.00475 mm.

This large, well-known *Opalina* has the characteristic form shown in the figures. Occasional anterior daughter cells, fresh from transverse division, are less slender behind. The more slender forms shown in the figures are probably recent products of longitudinal fission. The nuclei are large, being almost as large as in *Zelleriella microcarya*, which has the smallest nuclei of the known binucleated species.

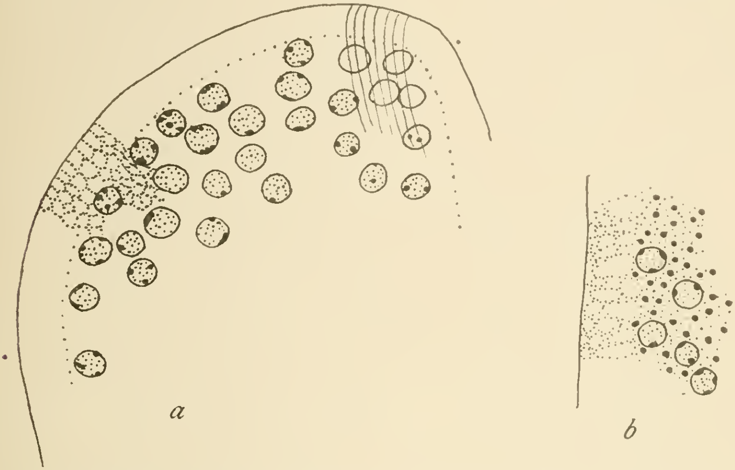


FIG. 142, *A*.—OPALINA OBTRIGONA, × 460 DIAMETERS: *a*, ANTERIOR END. THE FINE PARALLEL LINES REPRESENTING LINES OF INSERTION OF CILIA; *b*, A BIT OF THE SIDE OF THE BODY SHOWING COARSER ALVEOLATION OF THE ECTOSARC, THE ENDOSPHERULES, BEING DRAWN IN THIS FIGURE.

My material from the subspecies *japonica* and *savignyi* is not in good condition for detailed study. The infections in both cases seem to belong to the species *obtrigona*.

OPALINA OBTRIGONOIDEA, new species.

Type.—United States National Museum Cat. No. 16536.

Host.—*Bufo fowleri* Putnam, numerous living infections from Woods Hole, Massachusetts. The type infection was collected July 28, 1919, by M. M. Metcalf.

Measurements: A, of a large individual; B, of a small individual—

Measurements.	A	B
	mm.	*mm.*
Length of body	0.837	0.4
Width of body	.175	.18
Thickness of body	.021	.025
Diameter of nucleus	0.003- .005	0.003- .005
Diameter of endospherule	.00085	.00085

Opalina obtrigonoidea and its subspecies have a wide distribution from the whole Atlantic coast of North America to Alaska and Central America. It is likely that breeding experiments, if possible to carry out, would elevate to specific rank some of the forms here treated as subspecies, but their structure alone is not sufficient to demarcate them as species. The species *obtrigonoidea* is differentiated from *O. obtrigona* by its greater diversity in form, not only between different infections, but between different individuals in a

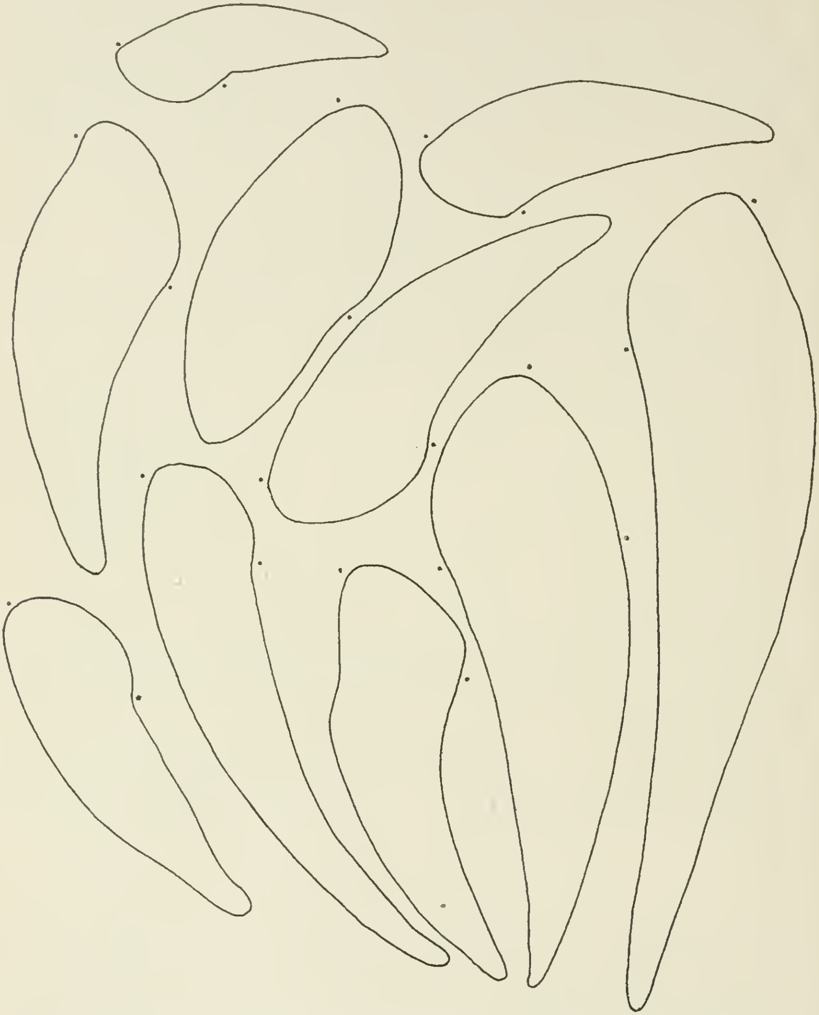


FIG. 143.—OPALINA OBTRIGONIDEA, FROM BUFO FOWLERI, × 117 DIAMETERS.

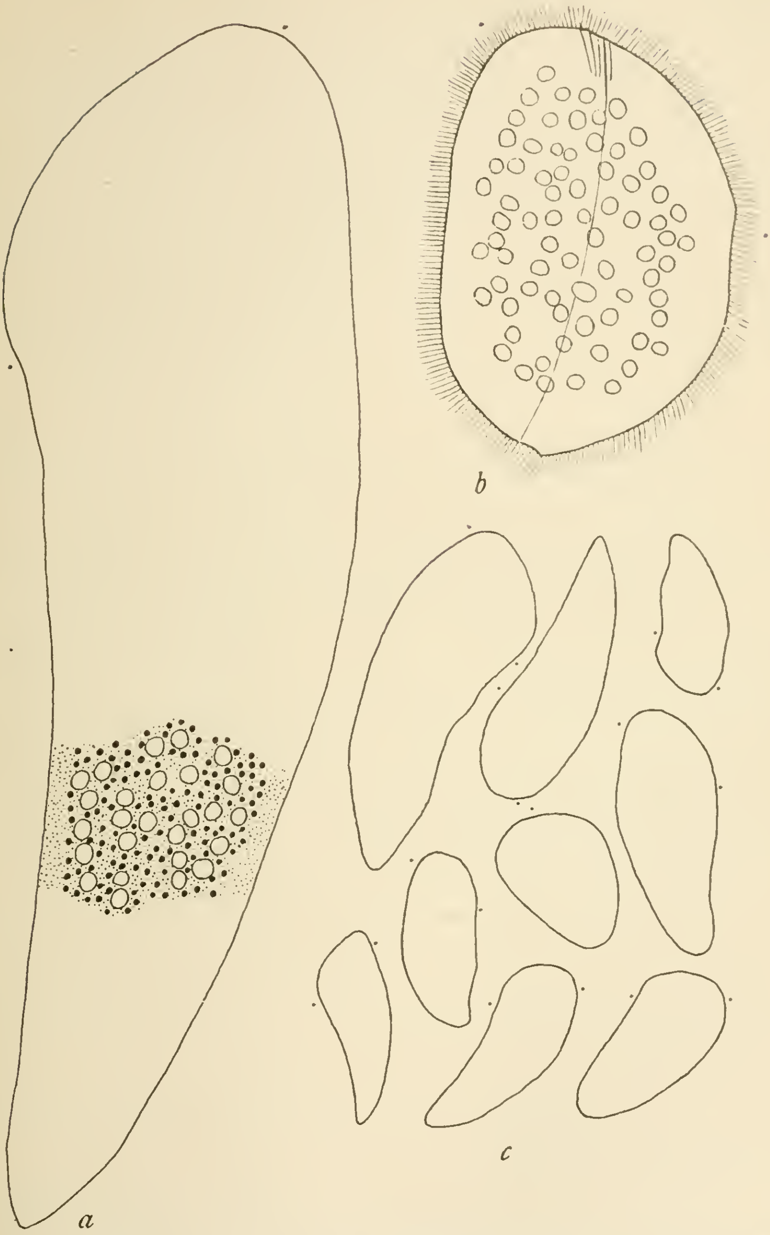


FIG. 144.—OPALINA OBTRIGONOIDEA, FROM SCAPHIOPUS SOLITARIUS: *a*, AND *b*, MAGNIFIED 460 DIAMETERS; *c*, A GROUP OF ANIMALS SHOWING RANGE OF SIZE AND FORM IN ONE INFECTION, × 117 DIAMETERS.

single infection, and by the small size of its nuclei. Note also the small endospherules in the list of measurements.

Opalina obtrigonoidea, United States National Museum No. 16581.

Host.—Scaphiopus solitarius Holbrook, numerous living infections from Raleigh, North Carolina, September, 1908; H. H. and C. S. Brimley, collectors.

Measurements of an ordinary individual.—Length of body 0.353 mm.; width of body 0.102 mm.; thickness of body, anterior, 0.0072 mm., middle 0.011 mm., posterior 0.01 mm.; diameter of nucleus 0.005 mm.; cilia line interval, anterior 0.00125 mm., middle 0.003 mm., posterior 0.003 mm.

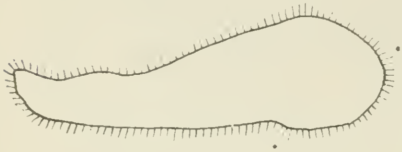


Fig. 145.—Opalina obtrigonoidea, from Bufo punctatus, × 117 diameters.

Opalina obtrigonoidea, United States National Museum specimen No. 16541.

Host.—Bufo punctatus Baird and Girard, one abundant infection, in United States National Museum specimen No. 26159, 41 mm. long, from Guanajuato, Mexico; A. Dugés, collector.

Measurements of a large individual.—Length of body 0.427 mm.; width of body 0.128 mm.; thickness of body 0.03 mm.; diameter of nucleus 0.0035 mm. to 0.0045 mm.; cilia line interval, anterior 0.0025 mm., middle 0.004 mm., posterior 0.0045 mm.

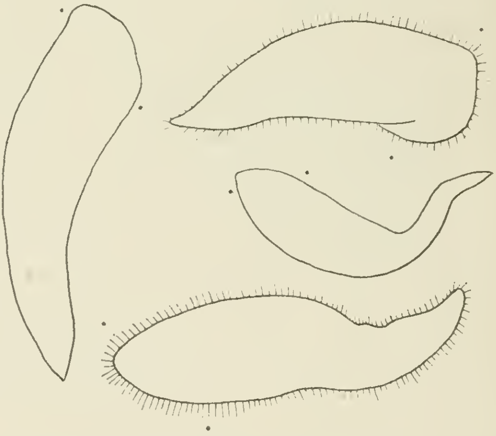


Fig. 146.—Opalina obtrigonoidea, from Hyla femoralis, × 117 diameters.

Opalina obtrigonoidea, United States National Museum No. 16542.

Host.—Hyla femoralis Daudín, four infections from Georgia. The specimen deposited in the United States National Museum is from United States National Museum specimen No. 5908, 32 mm. long, from Riceboro, "May or June"; LeConte, collector.

Measurements of a large individual.—Length of body 0.41 mm.; width of body 0.117 mm.; thickness of body 0.0325 mm.; diameter of nucleus 0.0045 mm. to 0.006 mm.; diameter of endospherule 0.002 mm.; cilia line interval, anterior 0.00175 mm., middle 0.004 mm., posterior 0.004 mm.

These Opalinas are like *O. obtrigonoidea*, but a good many of the individuals have the posterior end of the body more slender than we find it in any other infections of this species studied, and its nuclei are smaller. It is very like some of the smaller individuals from *Bufo fowleri*, but there is much less diversity of form than in the latter specimens.. It does not deserve treatment as a distinct subspecies.

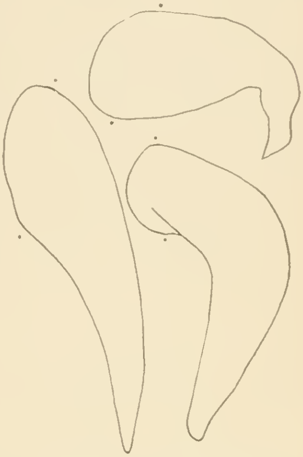


FIG. 147.—OPALINA OBTRIGONOIDEA, FROM HYLA ARENICOLOR, × 117 DIAMETERS.

Opalina obtrigonoidea, United States National Museum No. 16543.

Host.—*Hyla arenicolor* Cope, one abundant infection, from United States National Museum specimen No. 19726, 46 mm. long, from Fort Huachuca, Arizona, 1892; T. E. Wilcox, collector.

Measurements of an ordinary individual.—Length of body, 0.444 mm.; width of body, 0.12 mm.; thickness of body, anterior, 0.0385 mm., middle, 0.045 mm., posterior, 0.0385 mm.; diameter of nucleus 0.00475 mm. to 0.006 mm. (0.005 mm. mean); length and breadth of endospherule, first 0.0015 mm. by 0.00125 mm., second 0.00175 mm. by 0.0015 mm.; cilia line interval, anterior 0.00235 mm., middle 0.004 mm., posterior 0.004 mm.

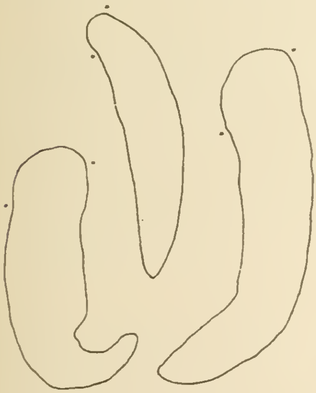


FIG. 148.—OPALINA OBTRIGONOIDEA, FROM HYLA PICKERINGII, × 117 DIAMETERS.

Opalina obtrigonoidea, United States National Museum No. 16544.

Host.—*Hyla pickeringii* (Holbrook), one abundant infection, in United States National Museum specimen No. 3604, 20 mm. long, from Aux Plains River, Illinois; R. Kennicott, collector.

Measurements of a large individual.—Length of body, 0.444 mm.; width of body, 0.09 mm.; thickness of body, anterior, 0.028 mm., middle 0.048 mm., posterior, 0.0358 mm.; diameter of nucleus, 0.004 mm.

to 0.006 mm. (mean 0.0043 mm.); diameter of endospherule, 0.00175 mm. to 0.0025 mm.; cilia line interval, anterior, 0.00183 mm., middle, 0.00375 mm., posterior, 0.00635 mm.

The cilia are unusually scant on the posterior part of the body of this *Opalina*, but the intervals between the lines of cilia are not, in general, dependable diagnostic characters, for they vary too much with the individual, but such an extreme interval between the cilia lines posteriorly, and such difference between the anterior and the posterior ends of the body in cilia line interval, is noteworthy.

Opalina obtrigonoidea, United States National Museum No. 16550.

Host.—*Gastrophryne carolinense* (Holbrook), one infection from Georgia, and another, the type, from United States National Museum specimen No. 48894, 28 mm. long, from Charlesburg, Virginia, July 22, 1910; F. P. Drowne, collector.

Measurements.—Length of body, 0.2 mm.; width of body, 0.085 mm.; diameter of nucleus, 0.0044 mm. to 0.0055 mm. (mean, 0.0047 mm.); diameter of endospherule, 0.0018 mm.; cilia line interval, anterior 0.00185 mm., posterior 0.00375 mm.

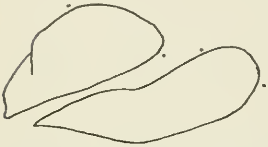


FIG. 149.—OPALINA OBTRIGONOIDEA, FROM GASTROPHRYNE CAROLINENSE, × 117 DIAMETERS.

Opalina obtrigonoidea, United States National Museum No. 16555, (figs. 150 and 151).

Host.—*Rana pipiens* Schreber, many infections, from Oberlin, Ohio, Raleigh, North Carolina, and Chicago, Illinois.

Measurements of a large individual.—Length of body, 0.453 mm.; width of body, 0.154 mm.; thickness of body, anterior, 0.0175 mm., middle, 0.018 mm., posterior, 0.019 mm.; diameter of nucleus, 0.0045 mm. to 0.0058 mm.; diameter of endospherule, 0.0022 mm. to 0.0026 mm.; cilia line interval, anterior, 0.0018 mm., posterior, 0.00287 mm.

Many of the individuals in these infections are broader than typical *Opalina obtrigonoidea* from *Bufo fowleri;* others are of the typical form.

Opalina obtrigonoidea, United States National Museum specimens Nos. 16560 and 16561 (figs. 152 and 153).

Host.—*Rana palustris* LeConte, many living infections from Woods Hole, Massachusetts, and from Oberlin, Ohio.

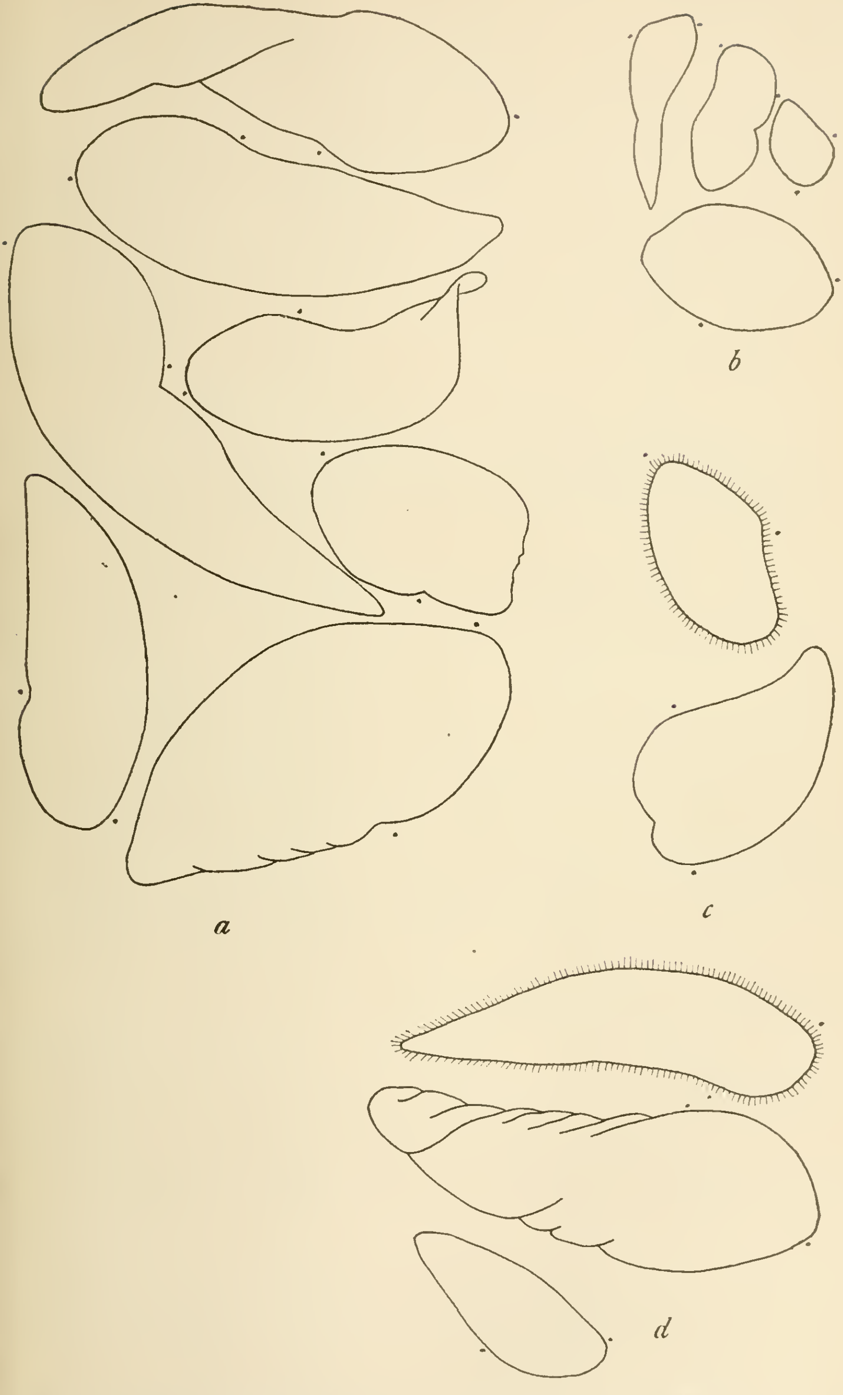


Fig. 150.—*Opalina obtrigonoidea*, from *Rana pipiens*, × 117 diameters: *a*, *b*, *c*, and *d*, groups of animals, each group from a different host.

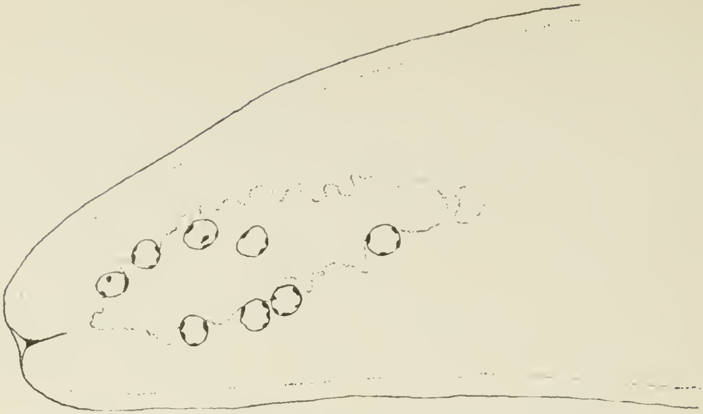


FIG. 151.—OPALINA OBTRIGONOIDEA, FROM RANA PIPIENS, OPTICAL SECTION SHOWING THE EXCRETORY PORE AND THE "BLADDER" OF THE EXCRETORY CANAL, × 1,000 DIAMETERS. THE TWO NUCLEI APPARENTLY LYING IN THE "BLADDER" ARE IN REALITY ABOVE IT.

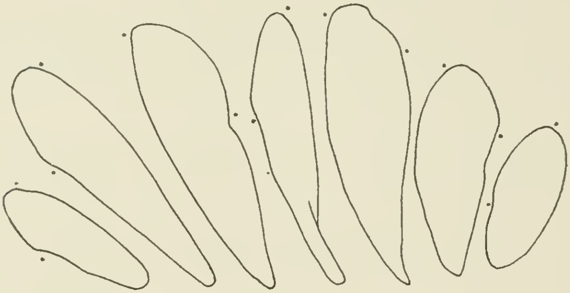


FIG. 152.—OPALINA OBTRIGONOIDEA, FROM RANA PALUSTRIS, × 117 DIAMETERS.

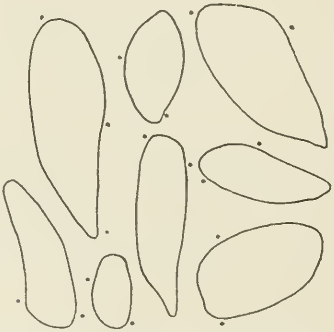


FIG. 153.—OPALINA OBTRIGONOIDEA, FROM RANA PALUSTRIS, SAMPLE INDIVIDUALS FROM ANOTHER HOST, × 117 DIAMETERS.

Measurements: A, of a large individual; B, of a small individual; C, of a plicate form—

Measurements.	*A*	*B*	*C*
	mm.	*mm.*	*mm.*
Length of body	0.325	0.0865	0.1
Width of body	.085	.05	.076
Thickness of body:			
Anterior	.013	.008	
Middle	.014	.013	
Posterior	.0125	.016	
Diameter of nucleus	0.004–.0048	0.00675–.0076	0.005–.0062
Cilia line interval:			
Anterior	.00187	.00118	
Posterior	.0033	.00275	

OPALINA OBTRIGONOIDEA, form PLICATA, new forma.

Type.—United States National Museum Cat. No. 16562.

Hosts.—*Rana palustris* LeConte, type infection, Woods Hole, Massachusetts, July 17, 1919; M. M. Metcalf, collector; also *Bufo fowleri* Putnam, from the same locality, summer 1919; M. M. Metcalf, collector; and another infection in this same host from Mount Monadnock, New Hampshire, August, 1912, M. J. Rathbun.

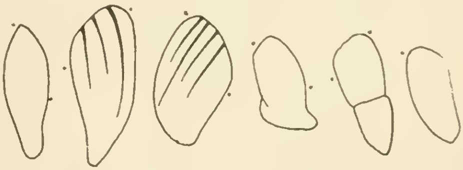


FIG. 154.—OPALINA OBTRIGONOIDEA, FROM RANA PALUSTRIS, SHOWING SOME INDIVIDUALS OF THE FORM PLICATA. × 117 DIAMETERS.

In some infections of *Opalina obtrigonoidea* we find many individuals which are thrown into longitudinal folds or ridges giving a quite distinctive appearance. A similar longitudinal plication is common in some other American Opalinas. I have not observed it in *Opalina obtrigona* in Europe.

OPALINA, species (?).

A specimen has been deposited with the United States National Museum as No. 16556.

Host.—*Rana septentrionalis* Baird, one scant infection, from United States National Museum specimen No. 13622, 45 mm. long, from Lucknow, Ontario, 1883; J. H. Garnier, collector.

Measurements of a large individual.—Length of body 0.23 mm.; width of body 0.0456 mm.; diameter of nucleus 0.004 mm. to 0.005 mm.

These forms resemble *O. obtrigonoidea* from *Rana pipiens*, but are too poorly preserved for description and classification.

OPALINA OBTRIGONOIDEA AMERICANA, new subspecies.

Type.—United States National Museum Cat. No. 16537.

Host.—*Bufo americanus* Holbrook, numerous living infections from Raleigh, North Carolina, April 14, 1912; H. H. and C. S. Brimley, collectors. Bufos of the same species, collected by the author in Oberlin, Ohio, have given Opalinids of this subspecies.

Measurements: A, of a large individual; B, of a small individual—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.94	0.246
Width of body	.186	.115
Thickness of body:		
Anterior	.021	.022
Middle		.026
Posterior	.0125	.025
Diameter of nucleus	0.005- .006	0.0065- .0077
Diameter of endospherule	.002	.002
Cilia line interval:		
Anterior	.0016	.0013
Posterior	.003	.00365

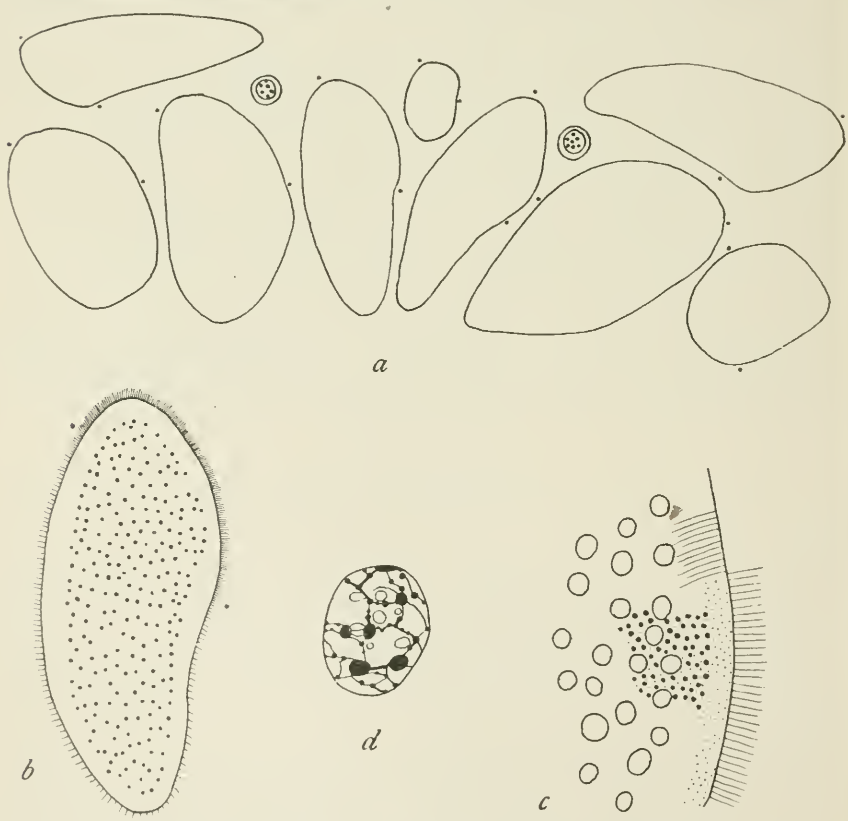


FIG. 155.—OPALINA OBTRIGONOIDEA AMERICANA, FROM BUFO AMERICANUS: *a*, A GROUP OF ANIMALS FROM ONE HOST, × 117 DIAMETERS; *b*, AN INDIVIDUAL WITH ALL ITS NUCLEI DRAWN, × 117 DIAMETERS; *c*, A BIT OF THE BODY, × 460 DIAMETERS; *d*, A NUCLEUS, × 2,000 DIAMETERS.

This subspecies, in my infections, has a much smaller proportion of long slender individuals than are found in infections of the species proper. Its nuclei are larger, especially in the smaller individuals. Its endospherules are much larger, and the intervals between the lines of cilia are narrower, though the latter is a feature of little diagnostic value.

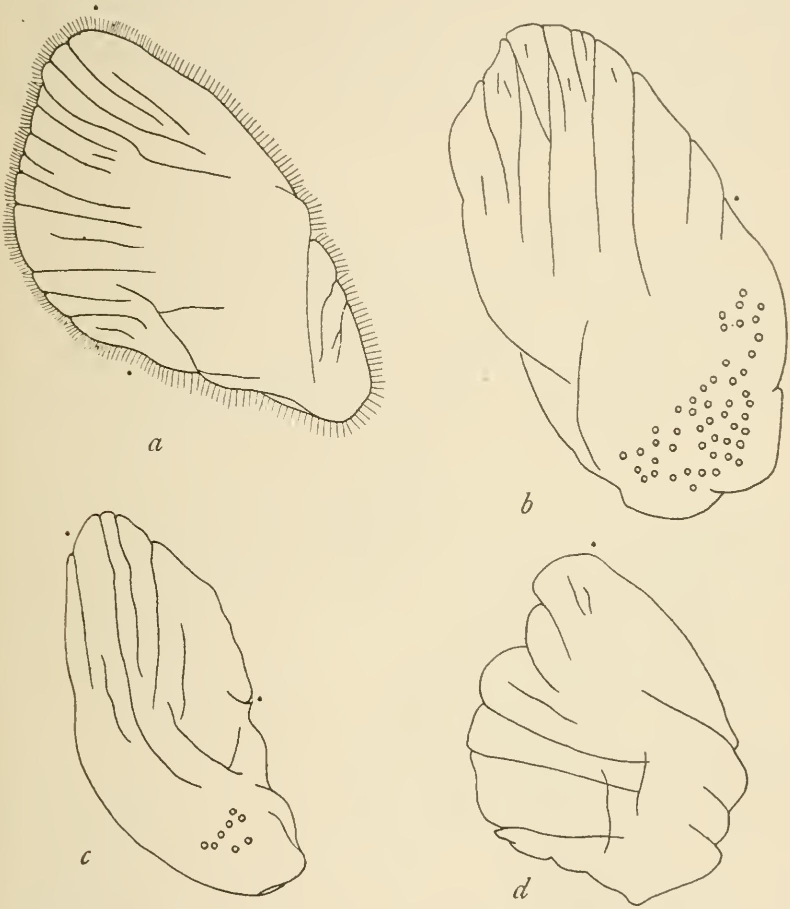


FIG 156.—OPALINA OBTRIGONOIDEA AMERICANA, FORM RUGOSA, FROM BUFO AMERICANUS, × 250 DIAMETERS; *d*, IS APPARENTLY AN ANTERIOR CELL FROM TRANSVERSE FISSION.

OPALINA OBTRIGONOIDEA AMERICANA, form RUGOSA, new forma.

Type.—United States National Museum Cat. No. 16584.

Host.—*Bufo americanus* Holbrook, 12 living infections from Raleigh, North Carolina, June, 1915; H. H. and C. S. Brimley, collectors.

Freshly taken infections often show their individuals shorter and broader than the usual *Opalina obtrigonoidea americana* and most of them crenate and rugose, as indicated in the figures. Such *rugosa* forms may be broad (length 0.276 mm., width 0.144 mm.) or narrower (length 0.222 mm., width 0.096 mm.). Nuclear and other conditions fail to differentiate the rugose forms from the subspecies proper. Since in numerous other species there are twisted, ridged, plicate or crenate individuals, and since the rugose condition and slightly shorter shape are all that distinguish the *rugosa* forms, it seems best not to recognize the rugose individuals as even a distinct variety, but to treat them rather as a *forma*, or possibly as only a condition which may be assumed at times under certain undetermined environmental circumstances.

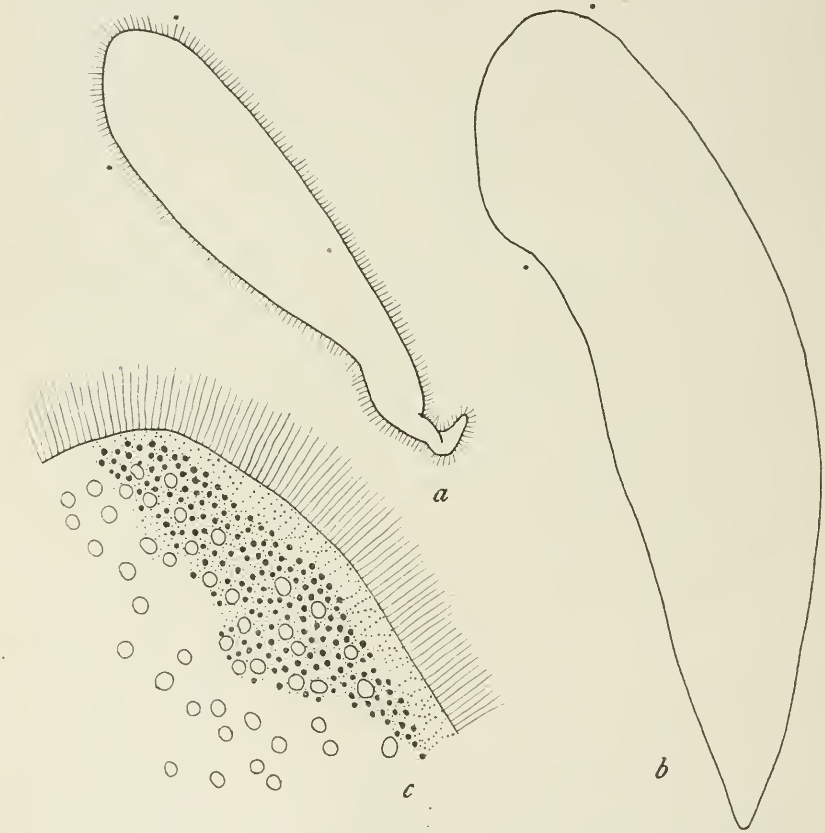


FIG. 157.—OPALINA OBTRIGONOIDEA MAXIMA, FROM BUFO BOREAS: *a* AND *b* MAGNIFIED 117 DIAMETERS; *c*, A BIT OF THE BODY MAGNIFIED 460 DIAMETERS.

OPALINA OBTRIGONOIDEA MAXIMA, new subspecies.

Type.—United States National Museum No. 16538.

Host.—*Bufo boreas* Baird and Girard, eight infections from western Canada and Alaska. The type infection is from United States National Museum specimen No. 50915, 72 mm. long, from Haines, Alaska, August 16, 1913, E. P. Walker collector.

Measurements.—

Measurements.	*A*	*B*	*C*
	mm.	*mm.*	*mm.*
Length of body	1.3	1.0	0.5
Width of body	.27	.245	.146
Thickness of body		.059	.049
Diameter of nucleus		.0035– .0055	Same.
Mean		.0048	
Diameter of endospherule		.0018– .0025	Same.
Cilia line interval:			
Anterior		.00225	Same.
Middle		.004	Same.
Posterior		.0045	Same.

This is the largest *Opalina* known. It is rivaled in bulk only by some of the Cepedeas which, though much shorter, are enough thicker almost to compensate. *Cepedea longa* is the only species approaching it in length and it is not nearly so broad. There is almost complete intergradation between these largest forms and *O. obtrigonoidea* from other hosts, so that the giant forms may be recognized only as a subspecies. It might even be better to treat them as a *forma.*

Opalina obtrigonoidea maxima. United States National Museum specimen No. 16614. (Figs. 158 and 159.)

Host.—*Bufo halophilus* Baird and Girard, many living infections from the vicinity of San Francisco, California, April, 1913; M. M. Metcalf collector.

Measurements.—

Measurements.	*A*	*B*	*C*	*D*
	mm.	*mm.*	*mm.*	*mm.*
Length of body	0.9	0.426	0.342	0.16
Width of body	.312	.113	.184	.08
Diameter of nucleus		.0048		.005
Cilia line interval:				
Anterior		.0015		
Middle		.002		.003
Posterior		.004		.004

These infections show greater diversity in size than those from *Bufo boreas*, some of the shorter ones seeming to be daughter cells recently come from transverse division. All the individuals in figures 158 and 159 are from a single slide from one infection. In a previous paper (Metcalf, 1914) I referred to the nuclear conditions in this *Opalina* from *Bufo halophilus*, saying that in my notes I was tentatively calling this form *O. intermedia*, because of its shape intermediate between that of *O. obtrigonoidea* and that of *O. ranarum*. Fuller comparison of all species indicates it is preferable to assign these forms to the species *obtrigonoidea*, recognizing the giant forms as a subspecies.

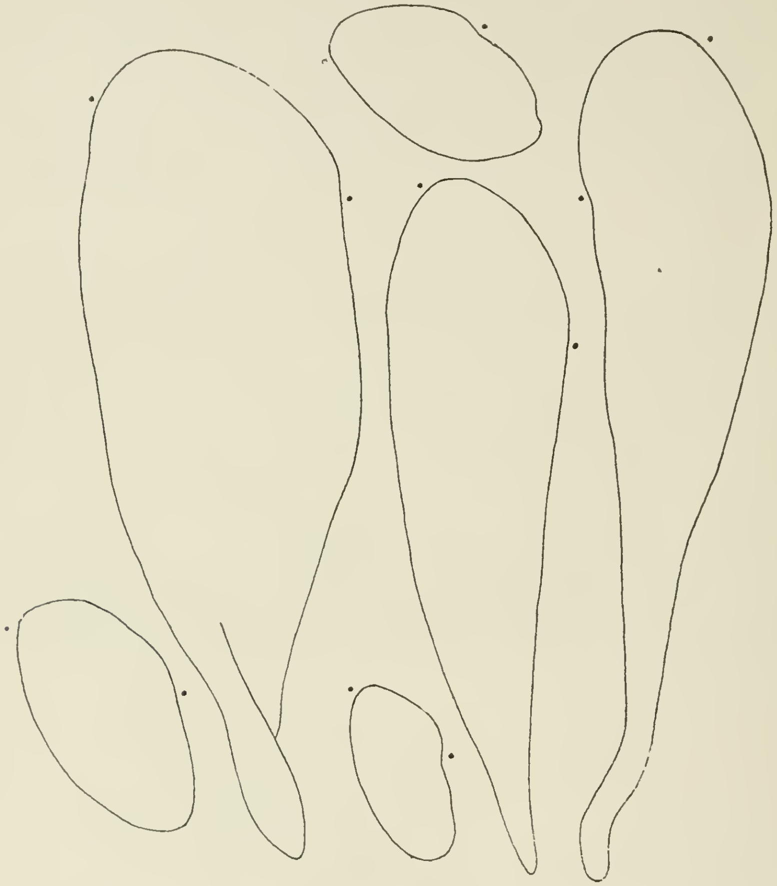


FIG. 158.—OPALINA OBTRIGONOIDEA MAXIMA, FROM BUFO HALOPHILUS. × 117 DIAMETERS.

OPALINA OBTRIGONOIDEA LATA, new subspecies.

Type.—United States National Museum Cat. No. 16563.

Host.—*Rana aurora* Baird and Girard, one infection, from United States National Museum specimen No. 39871, 58 mm. long, a female with eggs, from Crater Lake, Oregon, August 21, 1896; Everman and Cox collectors.

Measurements of a good sized individual.—Length of body 0.256 mm.; width of body 0.12 mm.; thickness of body, anterior 0.019 mm., middle 0.05 mm., posterior 0.04 mm.; diameter of nucleus 0.003 mm. to 0.004 mm. (dimensions of oval nuclei which seem to be entering on mitosis 0.0036 mm. by 0.0027 mm., 0.0045 mm. by 0.003 mm.); diameter of endospherule 0.002 mm. to 0.0022 mm.; cilia line interval, anterior 0.0018 mm. (second specimen 0.002 mm.), posterior 0.0028 mm. (the second specimen 0.00375 mm.).

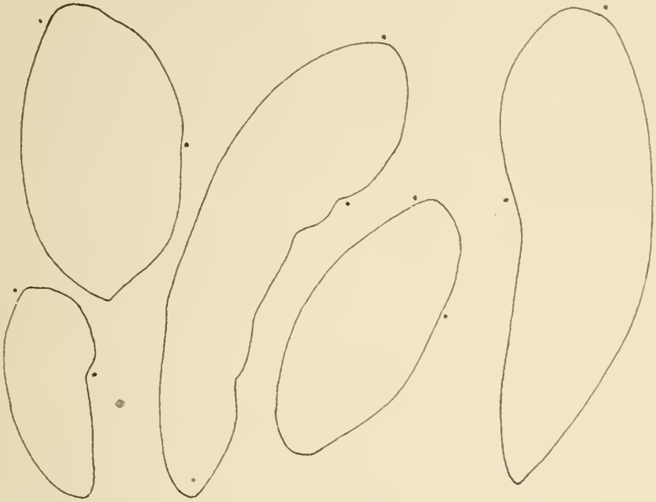


Fig. 159.—Opalina obtrigonoidea maxima, from Bufo halophilus, same individual from which were taken the animals shown in figure 158. These two figures together show the range of form and size in this one infection, × 117 diameters.

This infection shows numerous broad individuals and few, if any, so slender as the usual *O. obtrigonoidea*. The nuclei are small and mostly spherical, but some are ellipsoidal, these seemingly being about to enter upon mitosis.

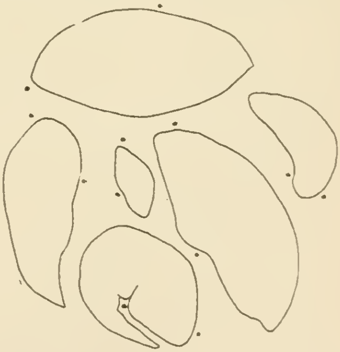


Fig. 160.—Opalina obtrigonoidea lata, from Rana aurora, × 117 diameters.

Opalina obtrigonoidea lata, United States National Museum specimen No. 16564.

Host.—Rana aesopus (Cope), one very abundant infection, from United States National Museum specimen No. 21703. 84 mm. long, from Crescent City, Florida, June 20, 1894; Henry G. Hubbard collector.

Measurements of a medium sized individual.—Length of body 0.236 mm.; width of body 0.09 mm.; thickness of body, anterior 0.01 mm., middle 0.018 mm., posterior 0.008 mm.; diameter of nucleus, 0.00375 mm. to 0.0048 mm.; diameter of endospherule 0.0015 mm. to 0.0022 mm.; cilia line interval, anterior 0.0019 mm., middle 0.0029 mm., posterior 0.0035 mm.

This infection shows some broad forms similar to those from *Rana aurora*, and it may well belong to the same subspecies. The nuclei run larger than those in the infection from the latter species.

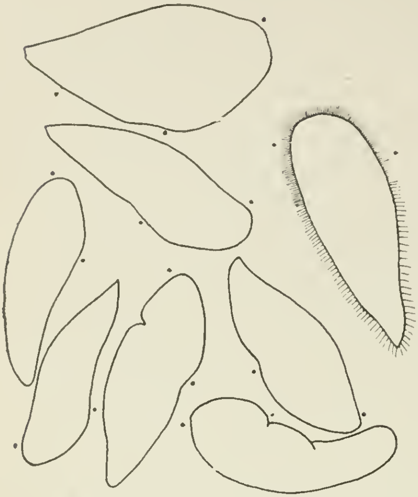


FIG. 161.—OPALINA OBTRIGONOIDEA LATA, FROM RANA AESOPUS, × 117 DIAMETERS.

OPALINA OBTRIGONOIDEA ORBICULATA, new subspecies.

Type.—United States National Museum Cat. No. 16565.

Host.—*Hyla cinerea* Schneider, five infections, from Texas, Louisiana, Florida, and Maryland. The type infection is from United States National Museum specimen No. 12005, 42 mm. long, from Georgiana, Florida; W. Wittfield, collector.

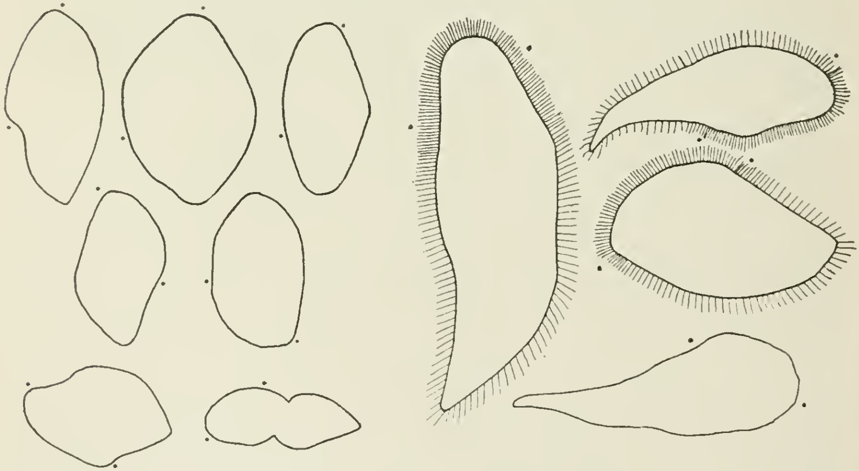


FIG. 162.—OPALINA OBTRIGONOIDEA ORBICULATA, × 117 DIAMETERS. THE EIGHT ANIMALS AT THE LEFT ARE FROM ONE HOST; THE FOUR AT THE RIGHT ARE FROM ANOTHER HOST.

Measurements: A, of a large, slender individual; B, of a smaller, broader form, both from Maryland—

Measurements.	A	B
	mm.	*mm.*
Length of body	.427	0.265
Width of body	.14	.145
Thickness of body	.031	.042
Diameter of nucleus		.0045–.005

Some of the individuals in these infections are *obtrigonoidea*-like in form, others are short and broad, approaching *O. ranarum* in shape. It is noteworthy that the endospherules in the Maryland infection are of diverse sizes, some minute, some very large. I do not understand this condition.

OPALINA OBTRIGONOIDEA AUSTRICOLA, new subspecies.

Type.—United States National Museum Cat. No. 16590.

Host.—*Rana pipiens austricola* (Cope), four infections, one from Porto Rico, one from Guatemala, and two from Tobasco, Mexico. Of the latter the type infection is from United States National Museum specimen No. 6506 (fig. 163, *b*), 38mm. long, collected March, 1864, by Doctor Berendt "in a well."

Measurements: A, of a large broad individual, an anterior daughter cell just come from transverse division (like the upper right figure in figure 163, b); B, of a long, narrower individual—

Measurements.	A	B
	mm.	*mm.*
Length of body	0.188	0.23
Width of body	.12	.09
Thickness of body	.045	....
Diameter of nucleus	.0032–.004	....
Most are	.004	....
Cilia line interval:		
Anterior	.0023	....
Posterior	.005	....

In another individual host of the same species, collected at the same time and place, are some much larger examples of this *Opalina* (fig. 163, *a*). A specimen has been deposited in the United States National Museum as No. 16592.

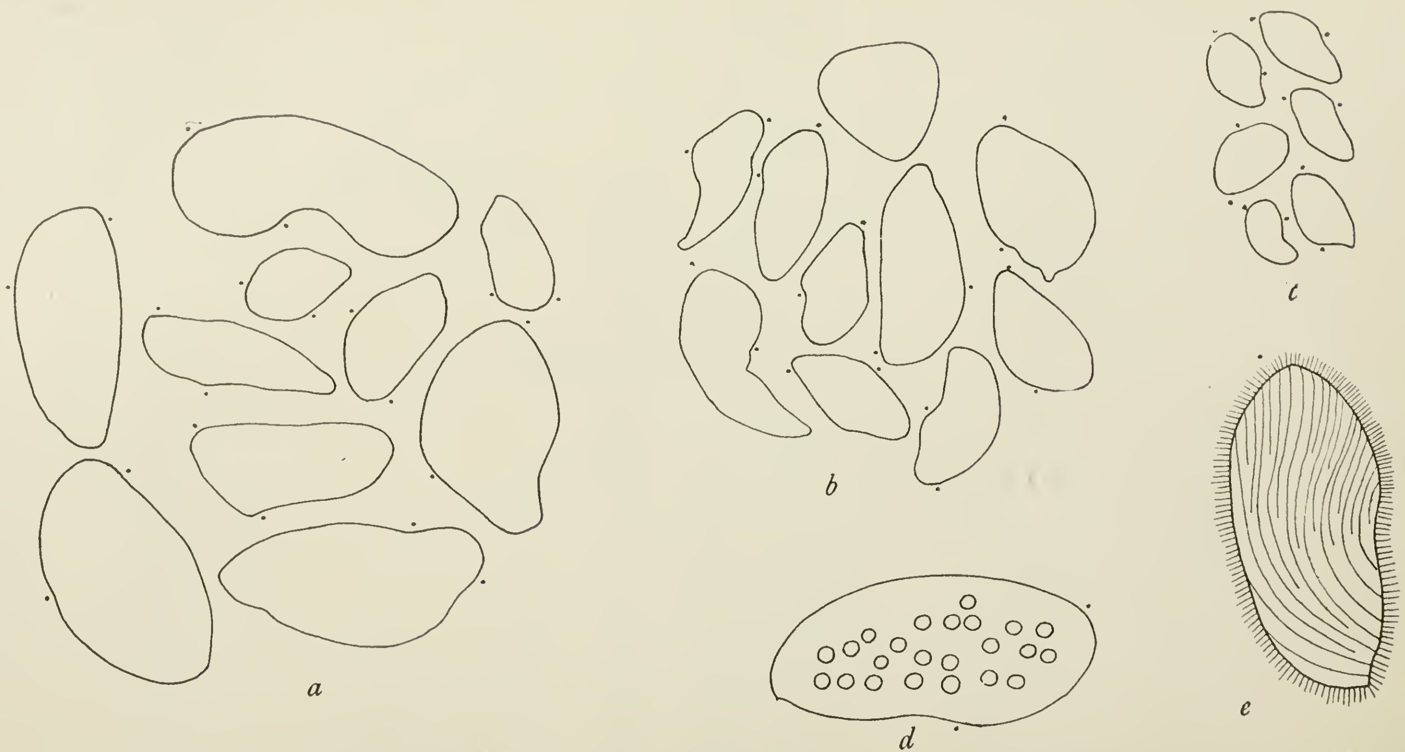


FIG. 163.—OPALINA OBTRIGONOIDEA AUSTRICOLA: *a*, *b*, AND *c*, GROUPS OF ANIMALS, EACH GROUP FROM A DIFFERENT INDIVIDUAL HOST, × 117 DIAMETERS; *d* AND *e* ARE ONE INDIVIDUAL FROM THE SAME HOST AS *c*, *d* SHOWING ALL THE NUCLEI, *e* SHOWING ALL THE LINES OF INSERTION OF THE CILIA ON ONE SURFACE OF THE BODY; *d* AND *e* ARE MAGNIFIED 460 DIAMETERS.

Measurements of A, a large individual, and B, a small individual from this infection are—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.3	0.17
Width of body	.17	.066
Thickness of body	.03	.03
Diameter of nucleus	.004– .0055	.0038– .005
Mean	.0042	
Diameter of endospherule	.0024– .0025	.0025
Cilia line interval:		
Anterior	.0019	
Middle		.003
Posterior	.00275	

The *Opalinae* in all these infections considerably resemble *O. obtrigonoidea lata*, and *O. obtrigonoidea orbiculata* and seem closely related.

Very much smaller *Opalinae* with larger nuclei are seen in a specimen of the same variety of frog, United States National Museum No. 30554. 27 mm. long, from Pico Blanco, Costa Rica, W. M. Gabb, collector (fig. 163, *c*, *d*, *e*). A specimen of these *Opalinae* has been deposited with the United States National Museum as No. 16591.

The measurements of a rather small individual are.—Length of body 0.096 mm.; width of body 0.0435 mm.; thickness of body 0.016 mm.; diameter of nucleus 0.005 mm. to 0.0065 mm., mean 0.0052 mm.; diameter of endospherule 0.0015 mm.; cilia line interval, anterior 0.00222 mm., posterior 0.0045 mm.

The largest individuals are only 0.117 mm. in length.

Noting the size of the three hosts mentioned and comparing the respective sizes of their *Opalinae*, one sees that the smallest (youngest) host has the smallest Opalinas, and the largest (oldest) host has the largest parasites, but the nuclei of the smallest Opalinas are much the largest. Whatever there may be in these conditions more than mere coincidence, we do not understand, for we do not know the details of structure throughout the life history of any multinucleated Opalinid. The study of these phenomena in detail would be of much value. Compare Hegner and Hsiang-Fong Wu (1921), published since this paper was ready for press, in which some details of growth are described for *O.* [*larvarum*].

OPALINA, species (?).

A specimen of this form has been deposited in the United States National Museum as No. 16611.

Host.—*Bufo haematiticus* Cope, one infection, from a United States National Museum specimen, one of many in a jar numbered

14181. The individual from which this infection was taken is 51 mm. long. It is from Nicaragua, August 7, 1895; J. F. Bransford, collector.

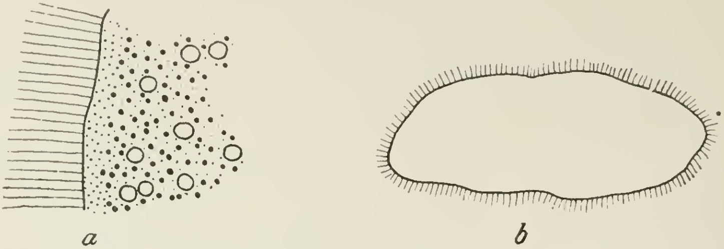


FIG. 164.—OPALINA, SPECIES?, FROM BUFO HAEMATITICUS: *a*, A BIT OF THE BODY MAGNIFIED 460 DIAMETERS; *b*, MAGNIFIED 117 DIAMETERS.

Measurements of two ordinary individuals—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.363	0.44
Width of body	.144	.107
Thickness of body	.025	.0255
Diameter of nucleus	.0044–.006	.003
Usual	.006	....
Cilia line interval, anterior	.00125	....

This is the third Opalinid found in *Bufo haematiticus.* We have already described *Zelleriella bufoxena* and *Cepedea dolichosoma.* This third species is quite flat. The material is none too good, nor is it abundant. It does not seem best to name it from this material. *Cepedea dolichosoma* was present in this individual host along with this unnamed *Opalina.*

OPALINA CAROLINENSIS, new species.

Type.—United States National Museum No. 16557. See also No. 16558.

Host.—*Rana pipiens sphenocephala* (Cope), two abundant infections; one, the type infection (fig. 165, *a*), from United States National Museum specimen No. 29003, 83 mm. long, from Kissimee Prairie, Florida, April 22, 1901, E. A. Mearns collector; the other (fig. 165, *b* and *c*) from Charleston, South Carolina.

Measurements: A, of a large individual; B, of a small individual—

Measurement.	*A*	*B*
	mm.	*mm.*
Length of body	0.4	0.089
Width of body	.17	.032
Thickness of body:		
Anterior	.026	.0105
Middle		.0105
Posterior	.04	
Diameter of nucleus	.005	0.0029-.0045
Diameter of endospherule	.002	.0012
Some are	0.0016×.0032	
Cilia line interval:		
Anterior	.00235	.0015
Middle		.0022
Posterior	.004	.0028

This *Opalina* resembles *O. obtrigonoidea*, but in most individuals the posterior end is acute, either tapering to a sharp point or abruptly acuminate.

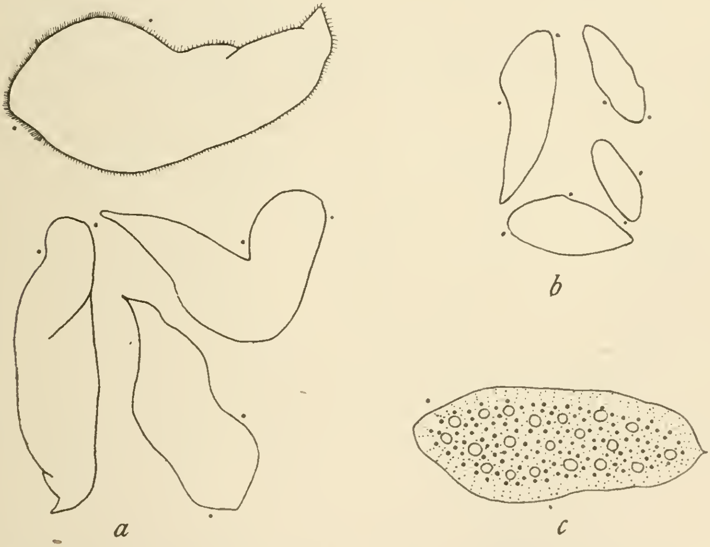


FIG. 165.—OPALINA CAROLINENSIS: *a* AND *b* FROM DIFFERENT INFECTIONS, × 117 DIAMETERS; *c* FROM THE SAME INFECTION AS *b*, MAGNIFIED 460 DIAMETERS.

OPALINA GUATEMALAE, new species.

Type.—United States National Museum No. 16567.

Host.—*Hyla baudinii* Duméril and Bibron, three infections from Guatemala, the type infection being from United States National Museum specimen No. 24819, 47 mm. long, collected by H. Hogue.

Measurements of a short, broad individual.—Length of body 0.24 mm.: width of body 0.132 mm.; thickness of body 0.023 mm.; diameter of nucleus 0.003 mm. to 0.0035 mm., many ellipsoidal, 0.0035 mm. by 0.003 mm.; cilia line interval, anterior 0.00145 mm., posterior 0.0028 mm.

A few individuals of this *Opalina* are *obtrigona*-like in form, but most are wider. The infections have a quite different appearance from those of *O. obtrigonoidea*, nor does this form resemble *O. obtrigonoidea lata* or *O. obtrigonoidea orbiculata*.

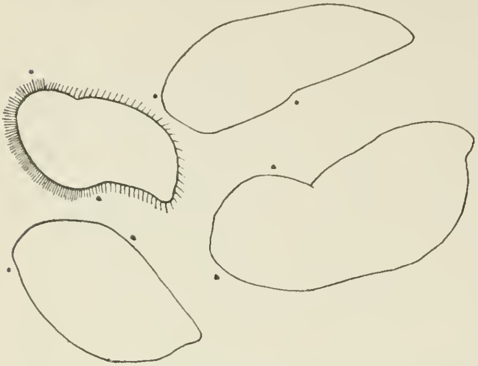


Fig. 166.—Opalina guatemalae, × 117 diameters. Three different infections are represented.

OPALINA WOODHOUSI, new species.

Type.—United States National Museum No. 16539.

Host.—*Bufo woodhousi* Girard, three infections, two from Arizona; the other, the type infection, from United States National Museum specimen No. 36364, 64 mm. long, from Utah, June 26, 1905.

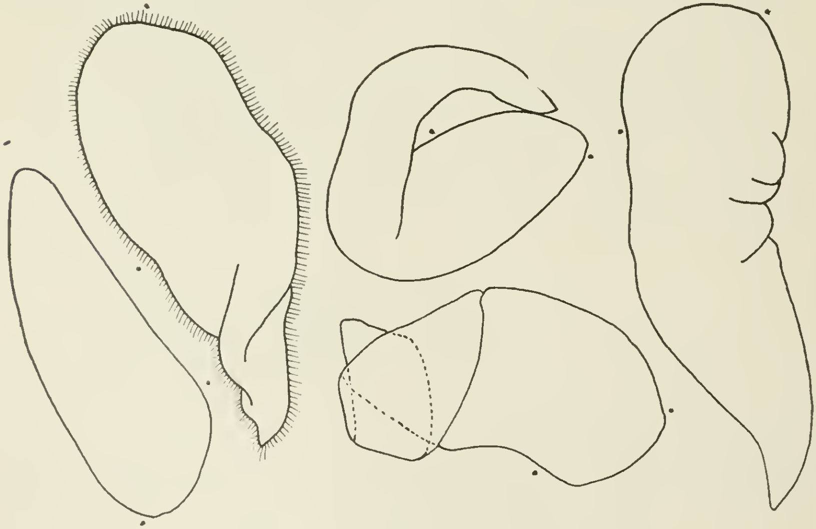


Fig. 167.—Opalina woodhousi, × 117 diameters.

Measurements of a large individual.—Length of body, 0.5.; width of body, 0.2 mm.; thickness of body, 0.023 mm.; length of nuclei, first 0.0065 mm., second 0.0071. mm.; width of nuclei, first 0.0046 mm., second 0.006 mm.; daughter cells with some almost spherical nuclei, 0.005 mm. by 0.0055 mm.; cilia line interval, anterior 0.00174 mm., posterior 0.0039 mm.

My infections of this species show less diversity of form than is seen in *Opalina obtrigonoidea.* The largest individuals are of about the same size as the smallest forms seen in infections of *O. obtrigonoidea maxima.* The point of chief difference from *O. obtrigonoidea* and its several subspecies is the elongated nuclei. In the resting condition they are distinctly ellipsoidal.

OPALINA DISCOPHRYA, new species.

Type.—United States National Museum Cat. No. 16585.

Host.—*Bufo cognatus* Say, one abundant infection, from United States National Museum specimen No. 37969, 89 mm. long, from Albuquerque, New Mexico, June 1, 1907; J. Hurter, collector.

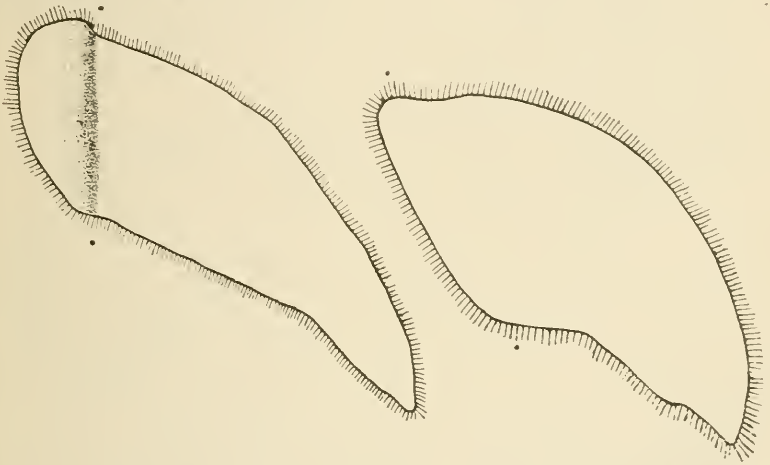


FIG. 168.—OPALINA DISCOPHRYA, FROM BUFO COGNATUS, × 117 DIAMETERS.

Measurements of an ordinary individual.—Length of body, 0.562 mm.; width of body, 0.18 mm.; thickness of body, 0.05.; diameter of nucleus, 0.0048 mm. to 0.0055: diameter of endospherule, 0.00137 mm.; cilia line interval, anterior 0.002 mm., middle 0.00375 mm., posterior 0.0041 mm.

Many individuals of this species have the anterior end of the body of peculiar form, somewhat resembling the anterior disk of *Discophrya cepedei*, a feature which suggests the specific name. Apparently in life the form of this thickened anterior end is various, for in the preserved specimens some individuals show the thickening greater than do others. In some cases the front of the body forms a flat, oval disk. In others there is merely a ridge a little behind and parallel to the anterior edge. In others there is no disk or ridge, merely an ordinary oblique anterior end. It would be desirable, of course, to see living animals before describing this feature of their form, but no living material has been available. The shape, however, is so characteristic as clearly to justify regarding this as a distinct species.

OPALINA DISCOPHRYA (?).

A specimen has been deposited with the United States National Museum as No. 16540.

Host.—Bufo copei Yarrow and Henshaw, six infections from northern and northeastern Canada: The type infection is from United States National Museum specimen No. 5388, from East Hudson's Bay; C. Drexler, collector.

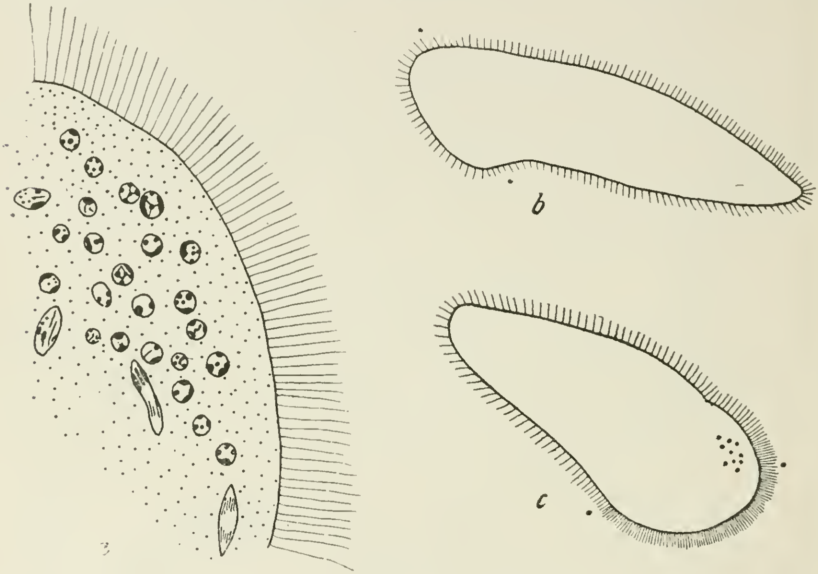


FIG. 169.—OPALINA DISCOPHRYA (?) FROM BUFO COPEI: *a*, A BIT OF THE BODY × 460 DIAMETERS; *b* AND *c*, MAGNIFIED 117 DIAMETERS.

Measurements of an ordinary individual.—Length of body, 0.4 mm.; width of body, 0.16 mm.; thickness of body, 0.03 mm.; diameter of nucleus, 0.00046 mm. to 0.00575 mm.; length of endospherule, 0.0025 mm.; width of endospherule, 0.002 mm.; cilia line interval, anterior, 0.0022 mm., middle, 0.0035 mm., posterior, 0.005 mm.

This infection from another *Bufo* from a very different locality shows in some individuals a shape similar to that of *O. discophrya* from *Bufo cognatus*, and its measurements quite agree, except the size and shape of the endospherules. It might be better to recognize it as a distinct subspecies.

OPALINA PICKERINGII, new species.

Type.—United States National Museum No. 16545.

Host.—Hyla pickeringii (Holbrook), numerous infections from Oberlin, Ohio, Raleigh, North Carolina, District of Columbia, and

Selkirk Settlement, Canada. The type infection was taken from a living frog from Raleigh, April, 1915; H. H. and C. S. Brimley collectors.

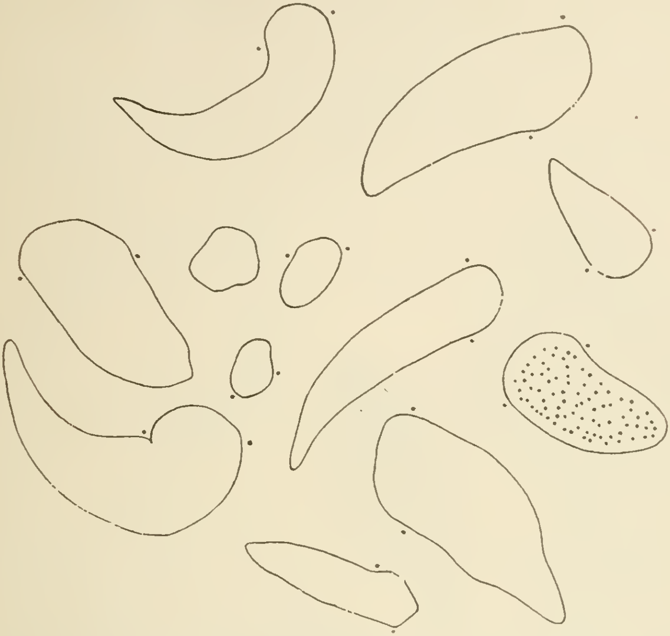


FIG. 170.—OPALINA PICKERINGII, × 117 DIAMETERS; ONE DRAWING SHOWS ALL THE NUCLEI IN THE INDIVIDUAL.

Measurements: A, of a short, broad individual; B, of a long, slender individual—

Measurements.	A.	B.
	mm.	*mm.*
Length of body	0.2	0.333
Width of body	.103	.068
Thickness of body:		
Anterior	.0208	.0158
Middle		.016
Posterior		.00168
Length and breadth of nuclei:		
First	0.005 × .004	....
Second	.0062 × .0045	....
Third	.007 × .005	....
Fourth	.0076 × .0065	....
Fifth	.0085 × .0066	....
Width and thickness of disk-shaped endospherule	.003 × .00125	....
Another	.0025 × .0015	....
Cilia line interval:		
Anterior	.0025	....
Middle	.0027	....
Posterior	.0038	....

There is much diversity of form in this *Opalina*. The individual measured as *A* is apparently an anterior daughter cell, short and posteriorly broad and rounded. Rather numerous comma-shaped forms are seen. The large, often very large, ellipsoidal nuclei and the large disk-shaped endospherules are noticeable characters.

OPALINA VIRGULA Dobell (1910).

Host.—Polypedates ["*Rhacophorus*"] *maculatus* (Gray), from Peradeniya Botanic Gardens, Ceylon, August; C. C. Dobell collector.

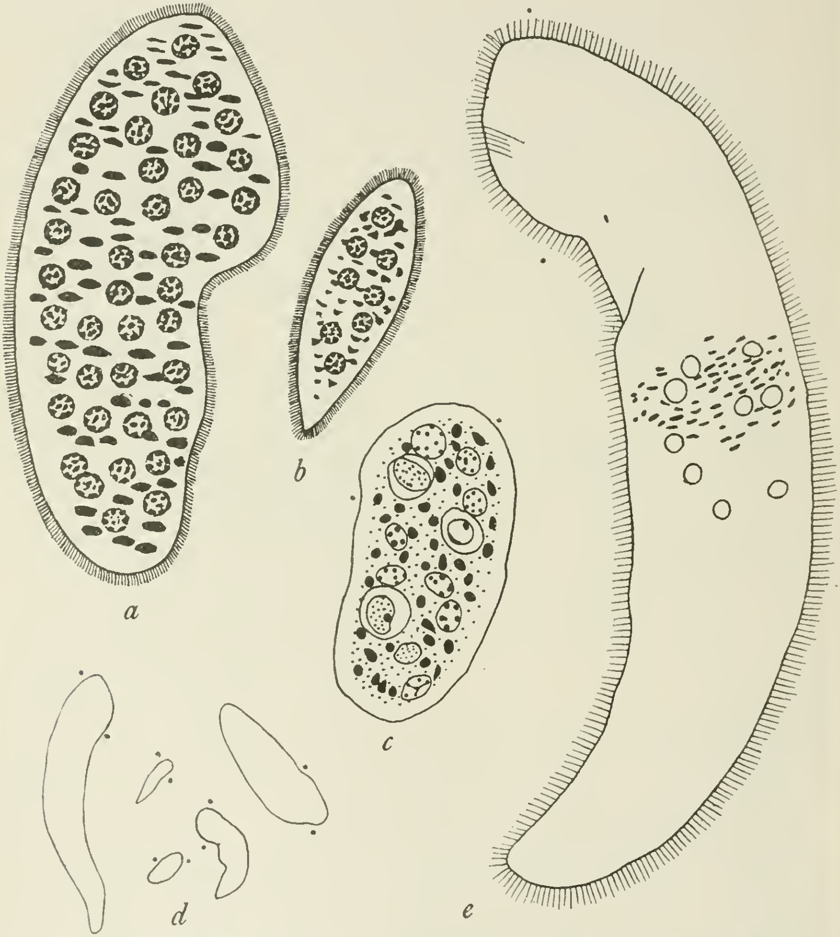


Fig. 171.—Opalina virgula: *a* and *b* from Dobell (1910); *c*, a very small individual magnified 1,000 diameters, all the nuclei present being shown; *d*, a group of animals from a single slide, × 117 diameters; *e*, an animal of usual character, × 460 diameters.

Professor Dobell very kindly sent me a slide of this interesting *Opalina* when he first described it, and the description here given is based upon this slide and upon Professor Dobell's paper.

Measurements of a good-sized individual of the slender form.—Length of body, 0.3 mm.; width of body, 0.085 mm.; thickness of body, 0.017 mm.; diameter of nucleus, 0.005 mm. to 0.0058 mm.; length, and width of endospherules, first 0.0032 mm. by 0.0012 mm., second 0.004 mm. by 0.001 mm., third 0.0042 mm. by 0.0014 mm.; cilia line interval, anterior, 0.00275 mm., posterior 0.00375 mm.

The measurements given by Dobell are different from the above.—Length of body, 0.17 mm., or rather over; width of body, 0.05 mm. at the broadest part of the anterior end; also smaller forms in the shape of a "flattened spindle," say, 0.038 mm. long by 0.013 mm. broad. Figures 171, *a* and *b*, are copied from Dobell's plate 2, figures 17 and 18, respectively. The animal figured by Dobell in his figure 17 is much more *obtrigona*-like than any I have found in the slide sent me.

This *Opalina* is defined by its general form, its large nuclei, and especially by its elongated, slender endospherules which lie with their long axes transverse to the length of the body and parallel to its surface, conditions different from that of any other known Opalinid.

There are in this species both long, slender forms and other broader forms. Some of the smallest individuals show degenerating nuclei and numerous smaller "secondary" (?) nuclei (fig. 171, *c*). The species seems clearly distinct. Considerations of geographical distribution and of structure have led me to question the relationship of this species to the *Opalinae*. (See p. 260.)

OPALINA VIRGULOIDEA, new species.

Type.—United States National Museum Cat. No. 16546.

Host.—*Hyla eximia* Baird, four infections, from Mexico. The type infection is from United States National Museum specimen No. 14601, September 1; A. Dugés collector.

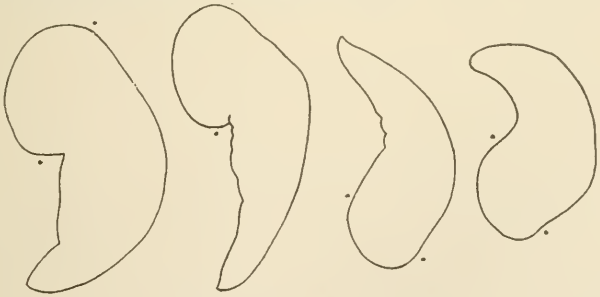


FIG. 172.—OPALINA VIRGULOIDEA, FROM HYLA EXIMIA, × 117 DIAMETERS.

Measurements of a large individual.—Length of body, 0.3 mm.; width of body 0.15 mm.; thickness of body 0.02 mm.; diameter of nucleus 0.0055 mm. to 0.0056 mm.; diameter of endospherule 0.0015 mm. to 0.0017 mm.; cilia line interval, anterior 0.0016 mm., middle 0.00175 mm., posterior 0.0025 mm.

Nearly all individuals in this species are much curved, with often an abruptly enlarged anterior end. They resemble in form the comma-shaped individuals of *O. pickeringii*, except that the latter are more slender. The nuclei are almost all spherical and run much smaller than those of *O. pickeringii.*

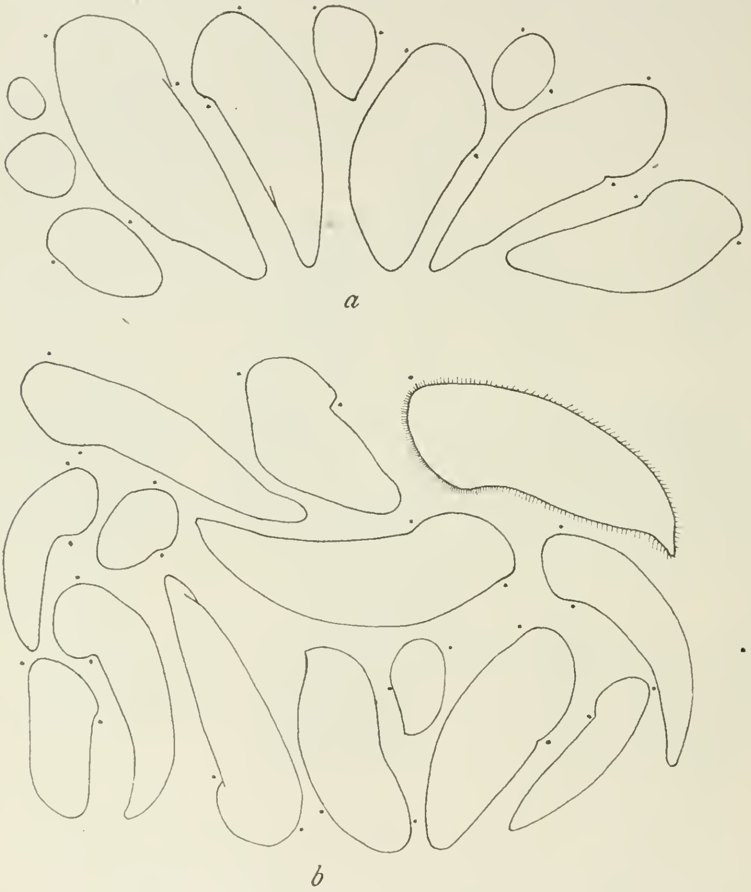


Fig. 173.—OPALINA VIRGULOIDEA, FROM RANA BOYLEI, × 117 DIAMETERS: *a* AND *b* ARE FROM DIFFERENT INFECTIONS.

Opalina virguloidea, United States National Museum No. 16615.

Host.—Rana boylei Baird and Girard, numerous living infections from frogs purchased in San Francisco, California, April 3, 1913, said to have been collected near San Francisco.

Measurements of a rather large individual.—Length of body, 0.34 mm.; width of body, 0.11 mm.; thickness of body, anterior 0.0105 mm., middle 0.0115 mm., posterior 0.013 mm.; diameter of nucleus

0.0048 mm. to 0.006 mm.; diameter of endospherule 0.0015 mm.; cilia line interval, anterior 0.00158 mm., posterior 0.005 mm.

These *Opalinae* grade into *O. virguloidea* from other hosts and can not be distinguished from them.

Opalina virguloidea, United States National Museum No. 16547.

Host.—*Hyla regilla* Baird and Girard, one abundant infection, from United States National Museum specimen No. 52321, 27 mm. long, from Los Angeles County, California; E. J. Brown, collector.

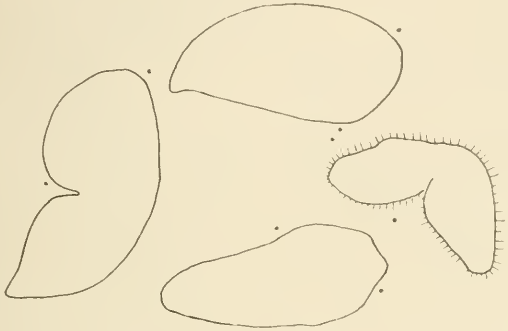


FIG. 174.—OPALINA VIRGULOIDEA, FROM HYLA REGILLA, × 117 DIAMETERS.

Measurements of two ordinary individuals—

Measurements.	*A.*	*B.*
	mm.	*mm.*
Length of body	0. 29	0. 3
Width of body	. 077	. 111
Thickness of body:		
Anterior		. 018
Posterior		. 0205
Diameter of nucleus	. 0055–. 007	0. 0053–. 0057
Width and thickness of disk-shaped endospherule	. 002 × . 001	.00175 × .00125
Second spherule	. 0025 × . 00125	
Cilia line interval:		
Anterior		. 00195
Middle		. 0032
Posterior		. 0035

This infection in *Hyla regilla* from southern California, as compared with infections in *Hyla eximia* from Mexico, gives quite a different impression. The diversity of form is greater in the California infections. Very few individuals show the exaggerated comma-shape seen in almost all individuals of the Mexican infections. There are numerous slender individuals. But the measurements of the California and the Mexican specimens agree, and those of the California individuals which are comma-shaped agree closely with the Mexican type. I am not attempting to distinguish the California forms from the others, though I confess to a feeling that they are not the same. Fuller series of infections from both hosts are needed to warrant any definite expression of opinion.

Opalina virguloidea, United States National Museum No. 16559.

Host.—Rana sylvatica LeConte, numerous living infections, from Oberlin, Ohio, and Raleigh, North Carolina. The type infection was collected at Oberlin April 28, 1915, by M. M. Metcalf.

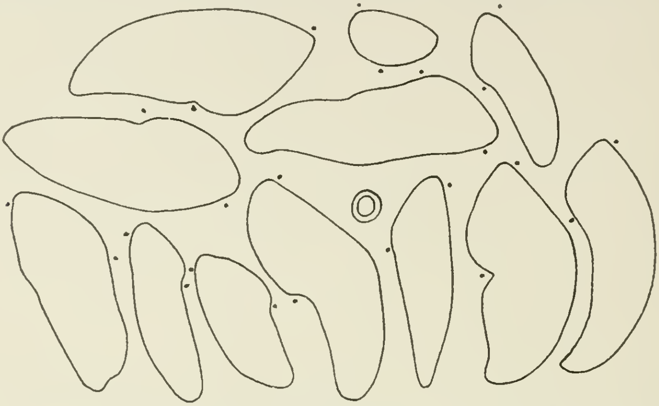


FIG. 175.—OPALINA VIRGULOIDEA, FROM RANA SILVATICA, × 117 DIAMETERS. THE SMALL CIRCULAR DRAWING REPRESENTS A CYST.

Measurements of an average individual.—Length of body, 0.273 mm.; width of body, 0.1 mm.; thickness of body, anterior 0.018 mm., middle 0.023 mm., posterior 0.024 mm.; diameter of nucleus, 0.005 mm. to 0.006 mm.; diameter of endospherule, 0.0015 mm. to 0.0018 mm.; cilia line interval, anterior 0.0019 mm., posterior 0.0041 mm.

OPALINA VIRGULOIDEA MAGNINUCLEATA, new subspecies.

Type.—United States National Museum No. 16549.

Host.—Acris gryllus (LeConte), numerous living infections, from North Carolina, H. H. and C. S. Brimley, collectors.

Measurements: A, of an average individual; B, of a large individual—

Measurements.	*A.*	*B.*
	mm.	*mm.*
Length of body	0.2	0.34
Width of body	.08	.13
Thickness of body	.0266	.025
Diameter of nucleus	0.006 .0075	0.00525 .0075
Diameter of endospherule		.0024
Cilia line interval:		
Anterior	.0025	.0018
Middle		.00262
Posterior	.00288	.004

Many individuals in these infections are comma-shaped, others are more *obtrigona*-like. The nuclei are much larger than in *O. virguloidea* from *Hyla eximia*. The best expression of the conditions observed seems to be to class these forms as a subspecies of *O. virguloidea*.

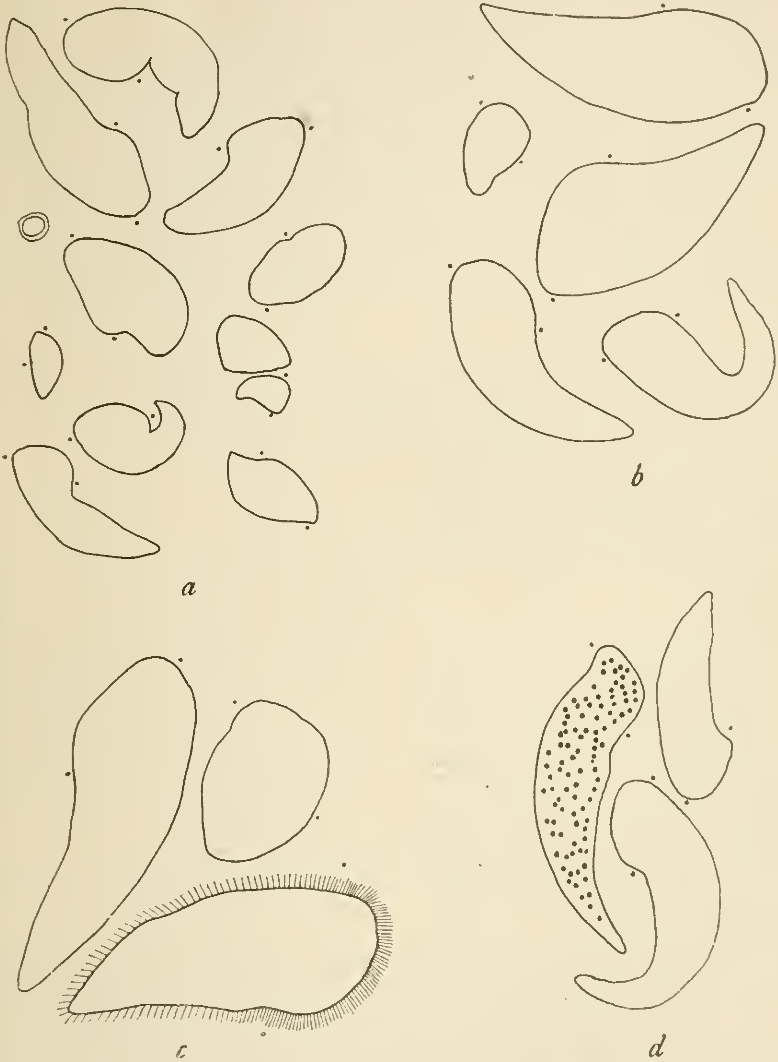


Fig. 176.—Opalina virguloidea magninucleata, × 117 diameters. Each group of figures is from a different individual host and shows the range of size and form in its infection. One drawing in group *d* shows all the nuclei in the cell.

OPALINA OREGONENSIS, new species.

Type.—United States National Museum Cat. No. 16548.

Host.—*Hyla regilla* Baird and Girard, two abundant infections from Vancouver Island, and another, the type infection, from United States National Museum specimen No. 51905, 40 mm. long, from Three Sisters, Oregon, 3,000 feet elevation, July 17, 1914; V. Bailey, collector.

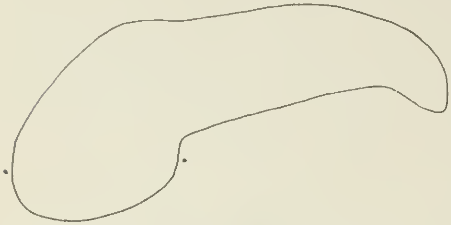


FIG. 177.—OPALINA OREGONENSIS, × 117 DIAMETERS.

Measurements of a large individual.—Length of body 0.526 mm.; width of body 0.123 mm.; thickness of body 0.017 mm.; length and width of nuclei, first 0.005 mm. by 0.003 mm.; second 0.007 mm. by 0.004 mm., third 0.0075 mm. by 0.0032 mm.; cilia line interval, anterior 0.0014 mm., middle 0.0025 mm., posterior 0.003 mm.

This large species differs from *O. virguloidea*, from the same host, chiefly in having nearly all of its nuclei ellipsoidal.

OPALINA KENNICOTTI, new species.

Type.—United States National Museum Cat. No. 16551.

Host.—*Rana areolata* Baird and Girard, one abundant infection, from United States National Museum specimen No. 9386, 45 mm. long, from northern Illinois; R. Kennicott, collector.

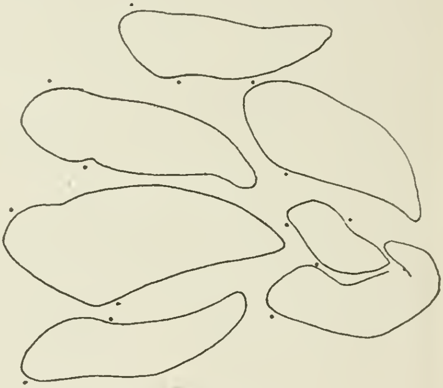


FIG. 178.—OPALINA KENNICOTTI, × 117 DIAMETERS.

Measurements of a medium sized individual.—Length of body 0.24 mm.; width of body 0.085 mm.; thickness of body, anterior 0.018 mm.. middle 0.027 mm., posterior 0.022 mm.; length and width of nuclei, first 0.00425 mm. by 0.00425 mm., second 0.0045 mm. by 0.0045 mm., third 0.0052 mm. by 0.003 mm., fourth 0.006 mm. by 0.0031 mm., fifth 0.00675 mm. by 0.0035 mm., sixth 0.0075 mm. by 0.0035 mm., seventh 0.0075 mm. by 0.0046 mm.; cilia line interval, anterior 0.0025 mm., posterior 0.00437 mm.

This *Opalina* is intermediate in form between *O. obtrigonoidea*, and *O. virguloidea.* A few of its nuclei, doubtless daughter nuclei from recent division, are spherical or nearly so. The rest are elongated, usually much so. The elongated nuclei distinguish it from species somewhat similar in form.

OPALINA TERRAE-MARIAE, new species.

Type.—United States National Museum No. 16568.

Host.—*Hyla evittata* Miller, one abundant infection, from United States National Museum specimen No. 32106, 43 mm. long, from Easton, Maryland, September 8, 1903, H. L. Clark, collector.

Measurements of an ordinary individual.—Length of body 0.177 mm.; width of body 0.133 mm.; thickness of body, anterior 0.01 mm., middle 0.03 mm., posterior 0.0217 mm.; diameter of nucleus 0.004 mm. to 0.00575 mm.; cilia line interval, anterior 0.0019 mm., middle 0.003 mm., posterior 0.0045 mm.

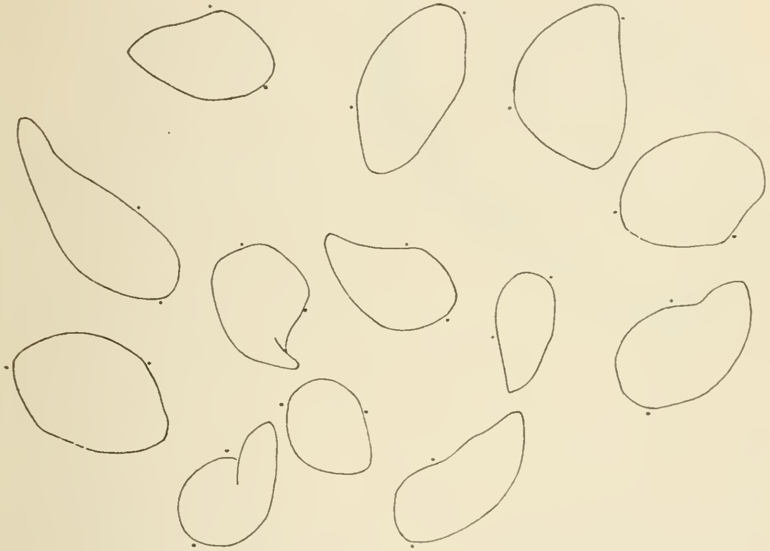


FIG. 179.—OPALINA TERRAE-MARIAE, × 117 DIAMETERS.

In spite of the short, broad form of most individuals of this species, it still seems to be related to the general *obtrigona* group, for some individuals show an *obtrigona*-like posterior end. It seems a distinct species.

OPALINA COPEI, new species.

Type.—United States National Museum No. 16586.

Host.—*Rana copei* Boulenger, two infections from Pico Blanco, Costa Rica, W. M. Gabb, collector; of these the type infection is from United States National Museum specimen No. 30654, 42 mm. long.

Measurements of a good-sized individual with 233 nuclei.—Length of body 0.24 mm.; width of body 0.135 mm.; thickness of body 0.015 mm.; diameter of nucleus 0.003 mm. to 0.0041 mm.; diameter of endospherule 0.002 mm.; cilia line interval, anterior 0.0025 mm., posterior 0.004 mm.

The broadest individuals of these infections seem almost *ranarum*-like in form, but others are more slender, approaching some of the shorter forms among the *obtrigona*-like species. It seems a distinct species, but not very sharply demarcated from related forms.

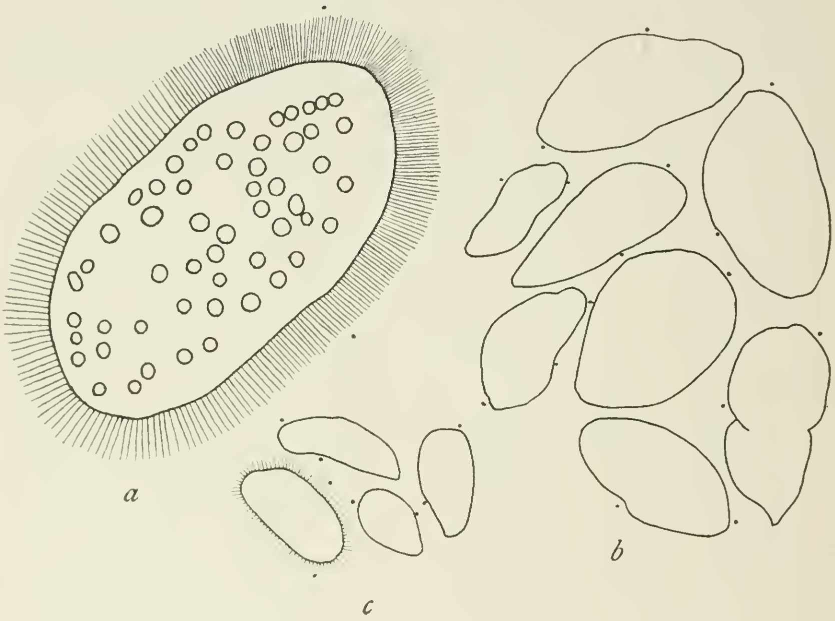


FIG. 180.—OPALINA COPEI, FROM RANA COPEI: *a*, MAGNIFIED 460 DIAMETERS; *b* AND *c*, SAMPLE ANIMALS SHOWING THE RANGE OF SIZE AND FORM IN EACH OF TWO INFECTIONS, × 117 DIAMETERS.

Opalina copei, United States National Museum specimen No. 16587 (fig. 181, p. 211).

Host.—*Rana pretiosa* Baird and Girard, three good infections, from Montana. The type infection is from United States National Museum specimen No. 17588, 72 mm. long, from Elliston, Montana, July 21, 1891; B. W. Evermann, collector.

Measurements of a large individual.—Length of body 0.273 mm.; width of body 0.13 mm.; thickness of body 0.0145 mm.; diameter of nucleus 0.004 mm. to 0.0055 mm.; diameter of endospherule 0.002 mm.; cilia line interval, anterior 0.0018 mm., posterior 0.00306 mm.

Though these infections are from a different species of host and from a very different locality, they seem to belong to *Opalina copei*.

Their nuclei run larger than in the latter species, but this may well be but a racial difference.

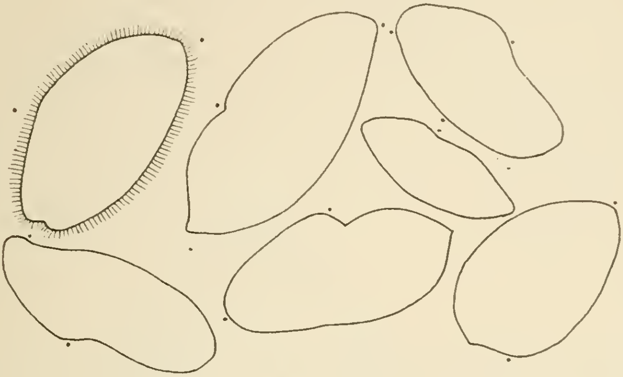


FIG. 181.—OPALINA COPEI, FROM RANA PRETIOSA, × 117 DIAMETERS.

OPALINA TRIANGULATA, new species (fig. 182, p. 212).

Type.—United States National Museum Cat. No. 16610.

Host.—*Bufo lentiginosus* Shaw, three infections, from Auburndale, Florida, March, 1912; N. R. Wood collector. The type infection is from United States National Museum specimen No. 48779. 64 mm. long.

*Measurements*s: *A, of a large individual; B, of a small individual*—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.32	0.1435
Width of body	.175	.063
Thickness of body	.033	.016
Diameter of nuclei	0.0034–.005	.00375
Mean	.004	....
Diameter of endospherule	.00125	.00125
Cilia line interval:		
Anterior	.00182	....
Middle	.0021	....
Posterior	.0039	....

This is another species whose broadest individuals approach in shape the *Opalinae latae.* It comes rather close to *O. copei*, also to *O. hylaxena*, from *georgiana*, but the general impression from the *triangulata* infections is quite different from that given by an infection of either of the forms mentioned. Some individuals of *O. panamensis* are of similar shape, but they have much larger nuclei, averaging one and one-half *micra* wider.

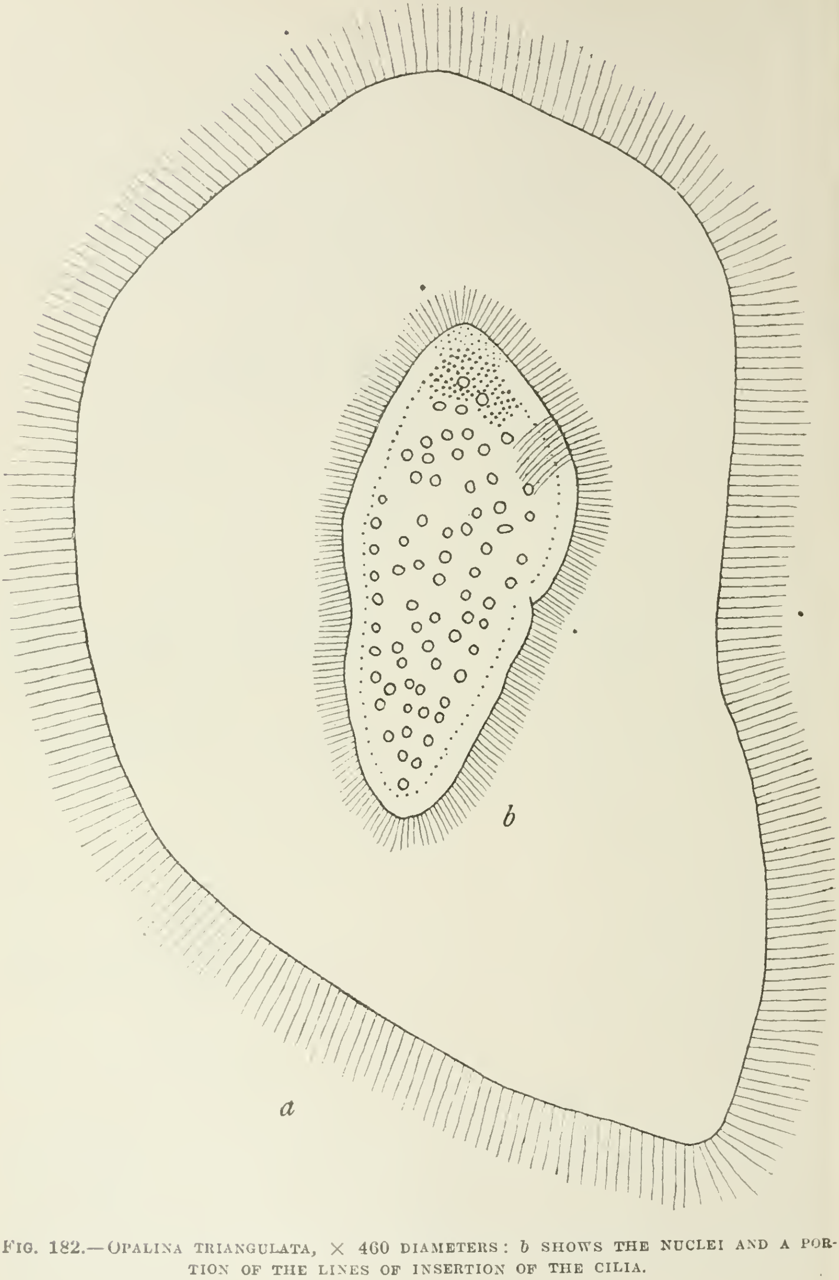


FIG. 182.—OPALINA TRIANGULATA, × 460 DIAMETERS: *b* SHOWS THE NUCLEI AND A PORTION OF THE LINES OF INSERTION OF THE CILIA.

OPALINA SPIRALIS, new species.

Type.—United States National Museum Cat. No. 16616.

Host.—*Bufo compactilis* Wiegmann, two abundant infections; one, the type infection, from United States National Museum specimen

No. 4964, 75 mm. long, from Pecos River, Texas; Captain Page, collector; the other from Prescott, Arizona.

Measurements: A, of a Texas specimen; B, of an Arizona specimen—

Measurements.	*A.*	*B.*
	mm.	*mm.*
Length of body	0.355	0.3
Width of body	.13	.14
Thickness of body	.025	.042
Diameter of nuclei (mean)	.0043	.0047
Diameter of endospherule	.00134	
Cilia line interval:		
Anterior		.00225
Posterior		.0031

The general appearance of these infections is quite different from that of *O. obtrigonoidea* or *O. virguloidea* infections, and the animals are not at all like *O. gigantea*, which also is found in *Bufo compactilis* from the same general locality. In both my infections most individuals have the posterior, slenderer portion of the body spirally plicated or twisted and this condition suggests the specific name. Of course, this is not a distinctive feature.

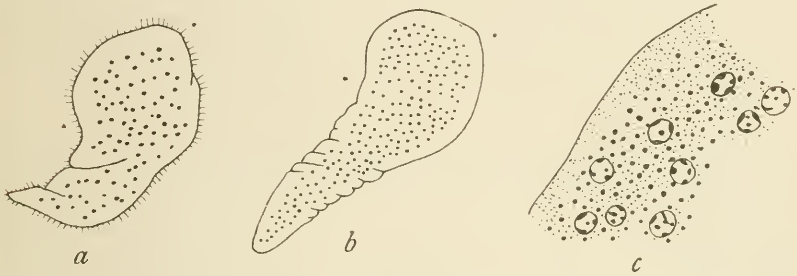


FIG. 183.—OPALINA SPIRALIS: *a* AND *b*, MAGNIFIED 117 DIAMETERS; *c*, A BIT OF THE BODY MAGNIFIED 460 DIAMETERS.

OPALINA CHOROPHILI, new species.

Type.—United States National Museum Cat. No. 16569.

Host.—*Chorophilus triseriatus* (Wied), many infections, from North Carolina and northern Ohio. The type infection is from a North Carolina frog collected by H. H. and C. S. Brimley in March, 1909.

Measurements of a large individual.—Length of body 0.47 mm.; width of body 0.1 mm.; thickness of body 0.03 mm.; diameter of nucleus 0.0035 mm. to 0.006 mm. (mean 0.005 mm.); cilia line interval, anterior 0.00106 mm., middle 0.0025 mm., posterior 0.0036 mm.

Note the ectosarc, very wide except at the anterior end of the body, where it is so narrow as hardly to be discernible.

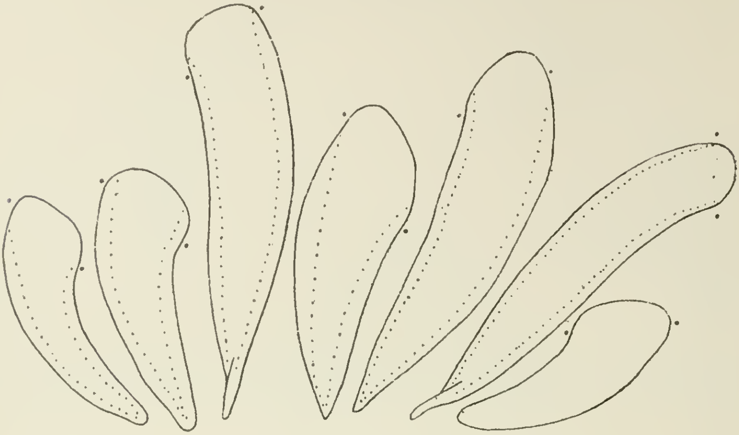


FIG. 184.—OPALINA CHOROPHILI, FROM CHOROPHILUS TRISERIATUS, × 117 DIAMETERS.

Opalina chorophili, United States National Museum specimen No. 16570.

Host.—Chorophilus occidentalis (Baird and Girard), two infections, one from Georgia, another (from which were taken the parasites deposited in the United States National Museum) from United States National Museum specimen No. 29189, 32 mm. long, from Hastings, Florida, June, 1901; Brimley, collector.

Measurements of a large individual.—Length of body 0.555 mm.; width of body 0.124 mm.; thickness of body 0.025 mm.: diameter of nucleus 0.00425 mm. to 0.0051 mm., mean 0.0045 mm.; length of endospherule 0.0015 mm.; width of endospherule 0.001 mm.; cilia line interval, anterior 0.0021 mm., middle 0.004 mm., posterior 0.004 mm.

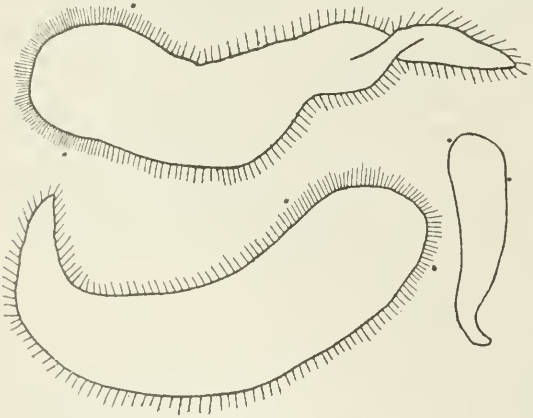


FIG. 185.—OPALINA CHOROPHILI, FROM CHOROPHILUS OCCIDENTALIS, × 117 DIAMETERS.

Opalina chorophili, United States National Museum No. 16571.

Host.—Chorophilus ornatus (Holbrook), two good infections, from Texas. The specimens of the parasite deposited with the United

States National Museum are from United States National Museum frog No. 15566, 35 mm. long, from Cook County, Texas; G. H. Roysdale collector.

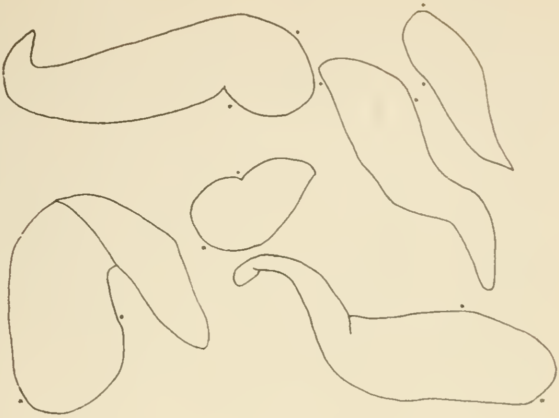


FIG. 186.—OPALINA CHOROPHILI, FROM CHOROPHILUS ORNATUS, × 117 DIAMETERS.

Measurements of a good-sized individual.—Length of body 0.41 mm.; width of body 0.1 mm.; thickness of body 0.031 mm.; diameter of nucleus 0.004 mm. to 0.0065 mm., mean 0.005 mm.; diameter of endospherule 0.002 mm. to 0.00225 mm.; cilia line interval, anterior 0.00207 mm., middle 0.00365 mm., posterior 0.00385 mm.

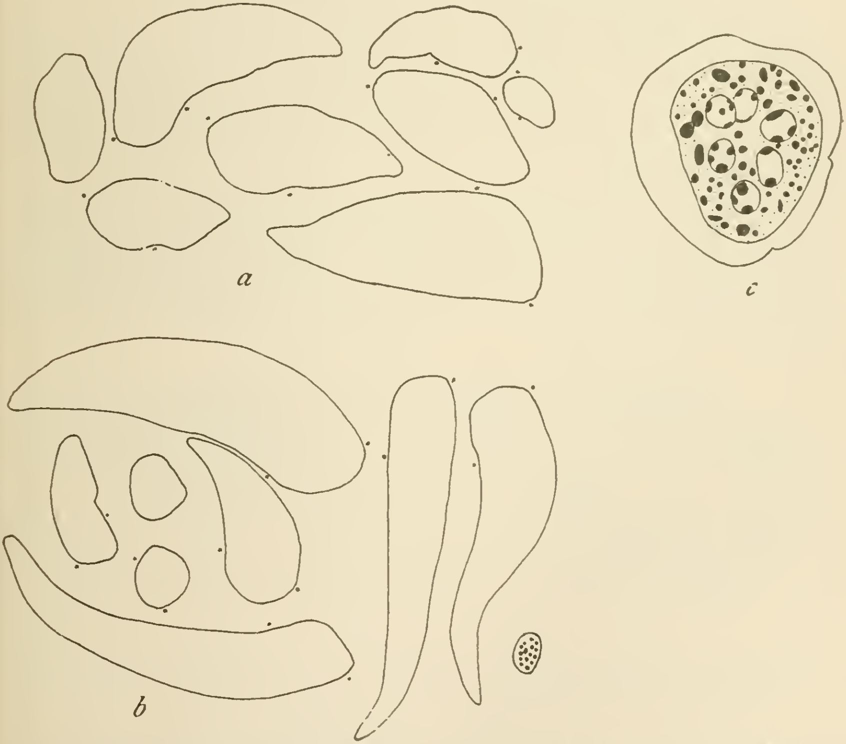


FIG. 187.—OPALINA CHOROPHILI, FROM CHOROPHILUS FERIARUM: *a* AND *b* SAMPLES FROM TWO INFECTIONS, SHOWING DIVERSITY IN SIZE AND FORM, × 117 DIAMETERS. (ONE MINUTE ANIMAL IN *b* SHOWS ITS NUCLEI); *c*, A CYST × 1,000 DIAMETERS.

Opalina chorophili, U. S. National Museum No. 16572 (fig. 187).

Host.—*Chorophilus feriarum* (Baird), four infections from United States National Museum frogs from North Carolina and the District of Columbia, and many living infections from Raleigh, North Carolina, April; H. H. and C. S. Brimley, collectors. From one of the latter infections were taken the specimens of the *Opalina* deposited with the United States National Museum.

Measurements of a long, slender individual.—Length of body 0.427 mm.; width of body 0.077 mm.; thickness of body 0.023 mm.; diameter of nucleus 0.004 mm. to 0.005 mm.; width and thickness of disk-shaped endospherule, first 0.003 mm. by 0.001 mm.; second 0.0025 mm. by 0.002 mm.; cilia line interval, anterior 0.00158 mm., middle 0.003 mm., posterior 0.0037 mm.

Opalina chorophili, from all its hosts, has a very wide ectosarc, except at the anterior end of the body. (Fig 183.) The slender posterior end of the body is usually more or less twisted. It seems to be distinct from *O. obtrigonoidea.*

OPALINA HELENAE, new species.

Type.—United States National Museum No. 16576.

Host.—*Agalychnis helenae* Cope, two infections, one from Nicaragua, and the other, the type infection, from United States National Museum specimen No. 29959, 65 mm. long, female with pale eggs 1.75 mm. in diameter, from San Carlos, Costa Rica; Burgdorf and Schild collectors.

Measurements of an average individual.—Length of body 0.4 mm.; width of body 0.1367 mm.; thickness of body 0.034 mm.; diameter of nucleus 0.0035 mm. to 0.0045 mm.; diameter of endospherule 0.0015 mm. to 0.00175 mm.; cilia line interval, anterior 0.00216 mm., middle 0.0035 mm.

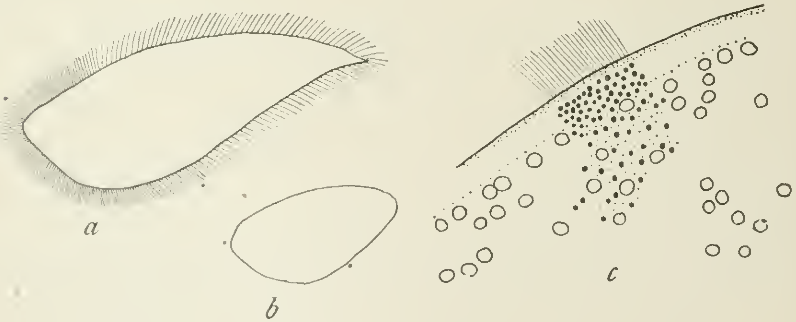


Fig. 188.—Opalina helenae: *a* and *b*, magnified 117 diameters; *c*, a bit of the body magnified 460 diameters.

There are in these infections wedge-shaped individuals, pointed at the posterior end, and wider forms. The cilia are long, though not sufficiently well preserved to be measured accurately. In figure c note the endospherules (?) invading the ectosarc in great number, a condition not understood. In many species a few endospherules may pass scatteringly into the ectosarc, but in no others has such wholesale invasion of the ectosarc been observed.

OPALINA HELENAE PHYLLOMEDUSAE, new subspecies.

Type.—United States National Museum Cat. No. 16578.

Host.—*Phyllomedusa dacnicolor* (Cope), two abundant infections from Mexico, the type infection being from United States National Museum frog No. 6037, 53 mm. long, Bischoff collector.

Measurements of an ordinary individual.—Length of body 0.3 mm.: width of body 0.16 mm.; thickness of body 0.046 mm.; diameter of nucleus 0.0031 mm. to 0.0036 mm.; diameter of endospherule 0.0017 mm. to 0.0022 mm.

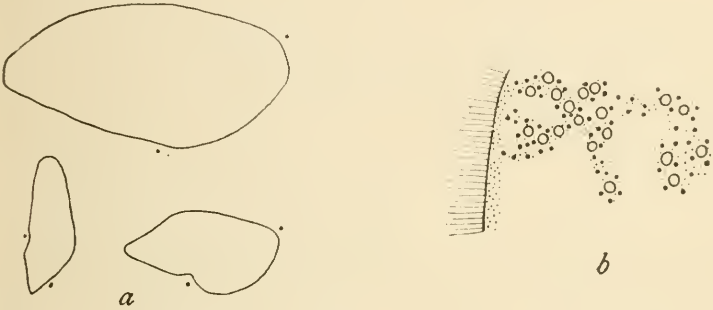


FIG. 189.—OPALINA HELENAE PHYLLOMEDUSAE: *a*, MAGNIFIED 117 DIAMETERS; *b*, A BIT OF THE BODY MAGNIFIED 460 DIAMETERS.

This *Opalina* is similar to *O. helenae*, but has shorter cilia and is not sharp-pointed posteriorly in any individual seen.

OPALINA MORELETEI, new species (fig. 190).

Type.—United States National Museum Cat. No. 16577.

Host.—*Agalychnis moreletei* (Duméril), two infections, from Guatemala, the type infection being from United States National Museum specimen No. 24827, 65 mm. long, collected by H. Hogue.

Measurements of a broad individual.—Length of body 0.287 mm.: width of body 0.182 mm.: thickness of body 0.016 mm.; diameter of nucleus 0.00225 mm. to 0.003 mm., mean 0.00275 mm.: diameter of endospherule 0.00075 mm.: cilia line interval, anterior 0.00171 mm., middle 0.003 mm., posterior 0.0041 mm.

There are broader and narrower forms in this species, but none are pointed behind. The nuclei are very small and the cilia are short. The endospherules are minute.

OPALINA HYLAXENA, new species.

Type.—United States National Museum Cat. No. 16573.

Host.—*Hyla versicolor* LeConte, one fine infection, alive, from a half-grown host, from Leland, Michigan, July 18, 1917; M. M. Metcalf, collector.

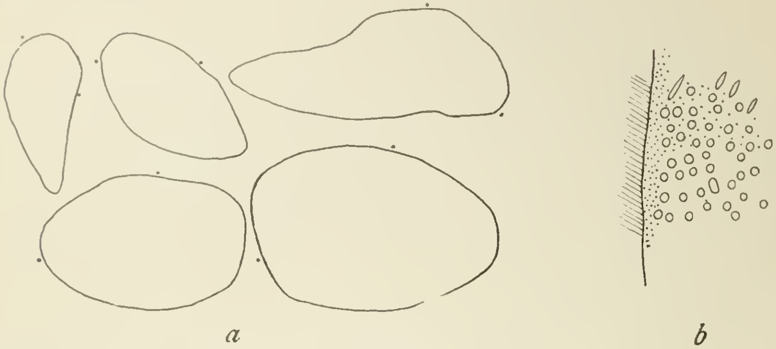


Fig. 190.—Opalina moreletii: *a*, magnified 117 diameters; *b*, a bit of the body × 460 diameters.

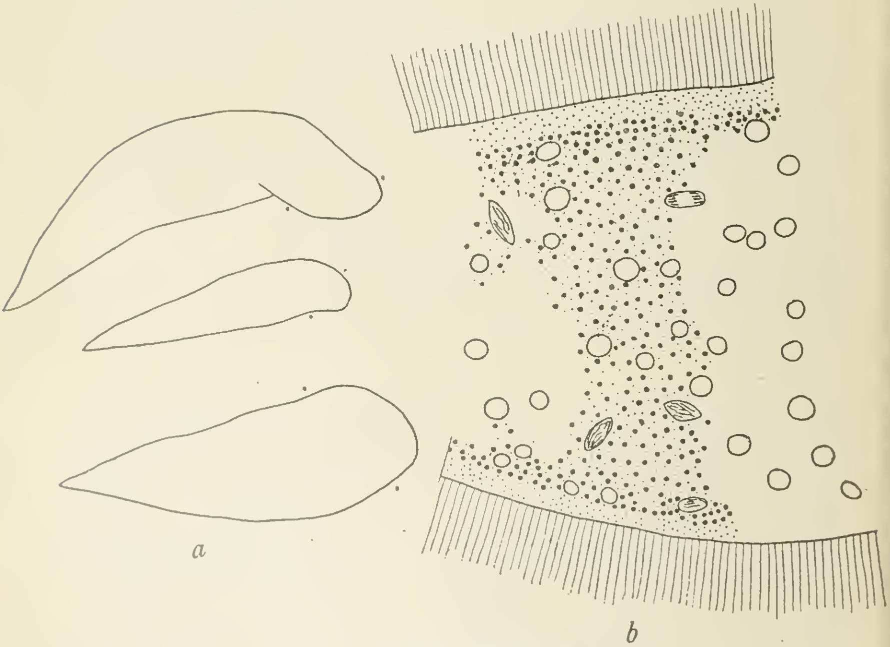


Fig. 191.—Opalina hylaxena: *a*, magnified 117 diameters; *b*, a portion of the body × 460 diameters.

Measurements of a good-sized individual.—Length of body 0.419 mm.; width of body 0.135 mm.; thickness of body, 0.0285 mm.; diameter of nucleus 0.0055 mm. to 0.008 mm.; diameter of endospherule, first 0.00126 mm., second 0.0015 mm., third 0.0016 mm.;

cilia line interval, anterior 0.00162 mm., middle 0.00275 mm., posterior 0.00275 mm.

The long and abundant cilia differentiate this species from *O. obtrigonoidea.* The nuclei are of very diverse size in my infections, due probably to the fact that division is taking place rather frequently, giving numerous small daughter nuclei and half-grown nuclei. The full-sized nuclei are large.

OPALINA HYLAXENA, form ORBICULATA, new forma.

Type.—United States National Museum Cat. No. 16575.

Host.—*Hyla versicolor* LeConte, many infections in tadpoles from Woods Hole, Massachusetts, July 21, 1919, collected for Dr. E. C. Just, who kindly gave them to me. All were found abundantly infected, but none of three adults of this tree frog from the same locality, which I opened, contained any Opalinas.

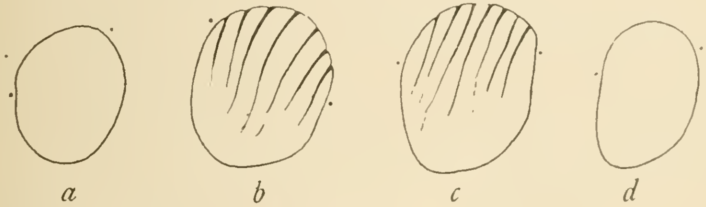


Fig. 192.—Opalina hylaxena form orbiculata. × 117 diameters; *c* and *d* indicate the longitudinal ridges seen in many individuals.

Measurements.—Length of body 0.205 mm.; width of body 0.154 mm.; diameter of nuclei 0.00755 mm. to 0.0085 mm.; diameter of nuclei of somewhat smaller individual 0.006 mm. to 0.0065 mm.

This very broad and short *Opalina* has very large nuclei. The animals are also quite thick. Most of the individuals are longitudinally plicate, the ridges being very noticeable both in life and in preserved specimens. I can not connect this form confidently with any species. These may be actively growing infections of *O. hylaxena*, short and broad because of the frequency of transverse division. The evidence pointing in this direction is the fact of residence in the same host and the further fact that the nuclei are very large, as in *O. hylaxena.* It seems best, at least tentatively, to class the Woods Hole forms as a *forma* or a possible subspecies of *O. hylaxena.*

OPALINA HYLAXENA, form PARVINUCLEATA, new forma.

Type.—By some mistake no specimens of this *forma* were kept. Endeavor will be made to obtain more material and, if successful, a type slide will be deposited in the United States National Museum.

Host.—*Hyla versicolor* LeConte, tadpoles, numerous infections from Woods Hole, Massachusetts, July, 1919; E. C. Just collector.

Measurements of a large individual.—Length of body 0.2 mm.; width of body 0.16 mm.; thickness of body, anterior 0.018 mm., middle 0.0248 mm., posterior 0.0219 mm.; diameter of nuclei 0.004 mm. (usual) to 0.00475 mm.; diameter of endospherules 0.0016 mm. to 0.00175 mm.; cilia line interval, anterior 0.0023 mm., middle 0.0036 mm., posterior 0.005 mm.

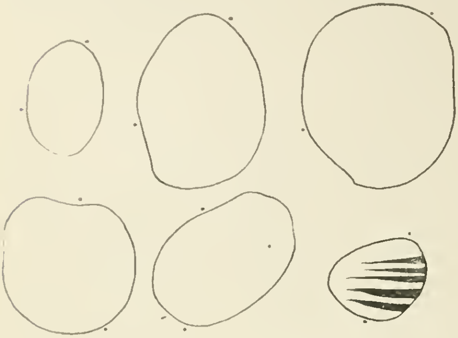


FIG. 193.—OPALINA HYLAXENA, FORM PARVINUCLEATA, × 117 DIAMETERS. THE HEAVY BLACK LINES IN THE LOWER RIGHT FIGURE INDICATE THE PLICATION OF THE BODY.

These Opalinas are of the same shape as *O. hylaxena*, form *orbiculata*, from the same lot of tadpoles, but their nuclei are very much smaller. Pending a fuller knowledge of the relation of nuclear size to physiological condition and phase of life cycle, it seems best to place the individuals of this sort as a *forma* under the species *hylaxena*.

OPALINA HYLAXENA, form GEORGIANA, new forma.

Type.—United States National Museum Cat. No. 16574.

Host.—*Hyla versicolor* LeConte, one infection, from United States National Museum specimen No. 44550, 45 mm. long, from Tate, Georgia, July 4, 1908; Howell, collector.

Measurements of a broad individual.—Length of body 0.192 mm.; width of body 0.162 mm.; diameter of nuclei 0.0031 mm. to 0.0045 mm., mean 0.004 mm.; diameter of endospherule 0.0016 mm. to 0.00175 mm.; cilia line interval, anterior 0.0015 mm.

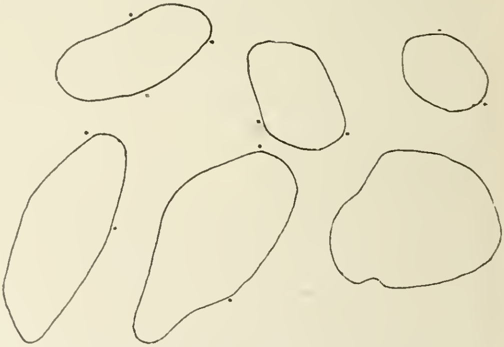


FIG. 194.—OPALINA HYLAXENA, FORM GEORGIANA, × 117 DIAMETERS. THE MORE MARKED IRREGULARITIES OF FORM ARE DUE TO DISTORTION FROM PRESSURE AT THE TIME THE SPECIMENS WERE KILLED IN THE RECTA OF THEIR HOSTS.

In this infection some individuals are as broad as the orbiculate and parvinucleate forms of *O. hylaxena*, while others are almost as narrow as the ordinary individuals of this species, but in all individuals the nuclei are small, as in the *forma parvinucleata*.

OPALINA, species (?).

A specimen of this *Opalina* has been deposited with the United States National Museum as No. 16579.

Host.—*Hyla versicolor chrysoscelis* Cope, two infections from New Braunfels, Texas; Lindheimer collector, both in poor condition.

Measurements.—The distorted bodies can not be measured with accuracy. The length and breadth of the nuclei range from 0.005 mm. by 0.007 mm. to 0.0062 mm. by 0.008 mm. The mean is about 0.005 mm. by 0.008 mm.

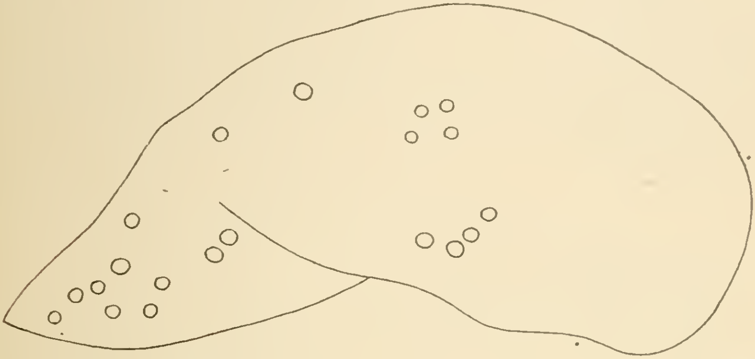


Fig. 195.—Opalina, species?, from Hyla versicolor chrysoscelis, × 460 diameters.

The species, of course, is not named from such material. A sample slide, from United States National Museum specimen No. 3234. 40 mm. long, has been deposited with this museum as Cat. No. 16579.

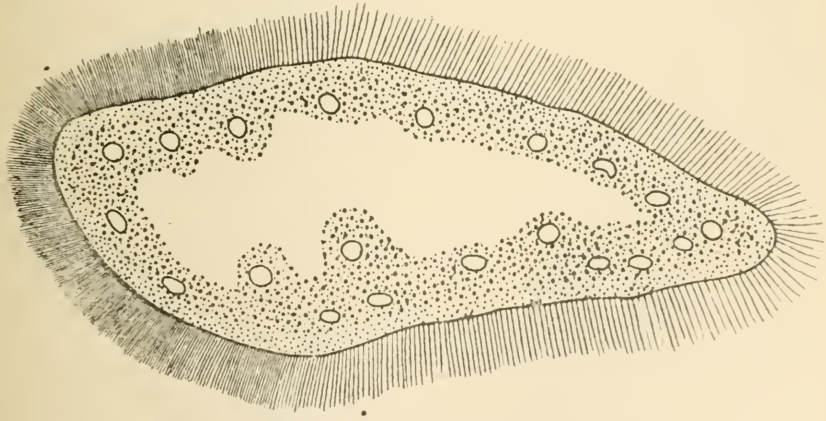


Fig. 196.—Opalina oblanceolata, × 460 diameters.

OPALINA OBLANCEOLATA, new species.

Type.—United States National Museum Cat. No. 16580.

Host.—*Scaphiopus hammondii* Baird, three infections, one from Chihuahua, Mexico, and two from Springerville, Arizona; of the latter, the type infection is from United States National Museum specimen No. 52144, collected August, 1914, by J. S. Ligon.

Measurements of an average individual.—Length of body 0.2 mm.; width of body 0.085 mm.; thickness of body 0.027 mm.; length and width of nuclei, first 0.005 mm. by 0.005 mm., second 0.006 mm. by 0.003 mm., third 0.007 mm. by 0.0045 mm.; cilia line interval, anterior 0.002 mm.

This *Opalina* shows a general resemblance to *Cepeda obovoidea*, but is much flatter, is not so pointed behind, and has most of its nuclei ellipsoidal, or perhaps discoid, instead of spheroidal. In my specimens the axial excretory organ is very large. This species is also very similar to many individuals of *Opalina obtrigonoidea.*

OPALINAE LATAE.

OPALINA RANARUM (Ehrenberg).

?*Chaos intestinalis cordiformis* Leeuwenhoek (1782).
?*Flimmerquadrata* Göze (1782).
Bursaria ranarum Ehrenberg (1831).
Opalina ranarum Purkinje and Valentin (1835).

A thoroughgoing study of this common European species would be a valuable thing, studying the marked racial diversity of which there is clear evidence, and following several of the races through their life history with the most careful attention to structural detail. It seems a favorable species for such study because of its accessibility and abundance and its decided diversity. I have not material for such study of this or any other species, and am not delaying the publication of this taxonomic review of the family until I can have obtained and have studied such extensive series of infections from all phases of the life history of some species, though without such thoroughgoing study of one species one's judgment as to single infections or a small group of infections of any species is likely to be erroneous. Without such intensive study one can not properly evaluate the structural characters observed. My material of this *Ophalina* includes numerous infections from *Rana temporaria* and one from its variety *parvipalmata*, as well as infections of somewhat similar Opalinas from other Ranas from the same general region, and I will describe in some detail the conditions found. But this series of infections is insufficient for an adequate study.

The hosts from which *Opalina ranarum* has been frequently reported are *Rana temporaria* Linnaeus, *Bufo bufo* (Linnaeus) [*Bufo vulgaris* Laurenti], and *Bufo viridis* Laurenti [*Bufo variabilis* Pallas]. My material includes *ranarum*-like infections from all these species and from *Rana dalmatina* Fitzinger. *Opalina ranarum* is also reported from *Bombina bombina* (Linnaeus), very unusual;

Rana esculauta, Linnaeus, rare, perhaps temporary infection; *Triturus alpestris* (Laurenti), very rare.

Opalina ranarum, United States National Museum No. 16593.

Host.—*Rana temporaria* from Würzburg, Germany, May 7, 1907; M. M. Metcalf, collector.

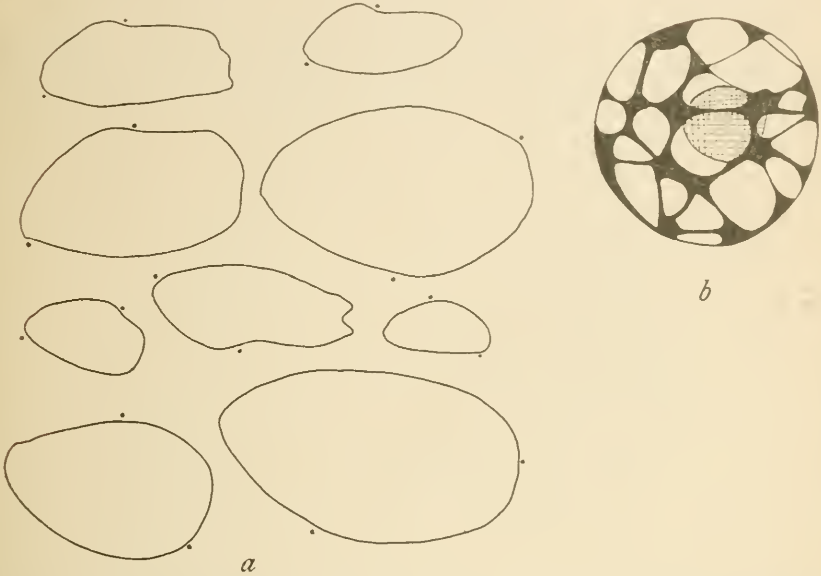


Fig. 197.—*Opalina ranarum*, from *Rana temporaria*: *a*, a group of animals, showing range of form and size, × 117 diameters; *b*, a nucleus × 4,100 diameters. The central body in *b*, conventionally shaded, is the nucleolus. Two. or perhaps three, of the drawings in *a* show individuals with irregular contour at the posterior end of the body, indicating that they are anterior daughter cells which have recently come from transverse fission.

Measurements: A, of alarge individual; B, of a medium-sized individual; C, of a small, narrow individual—

Measurements.	*A*	*B*	*C*
	mm.	*mm.*	*mm.*
Length of body	0. 277	0. 163	0. 145
Width of body	. 162	. 12	. 052
Thickness of body:			
Anterior	. 027	. 026	....
Middle	. 0255	. 027	....
Posterior	. 016	. 01	....
Diameter of nucleus	. 0065- . 0077	. 0065- . 0073	. 00475- . 0066
Diameter of endospherule	. 00225	. 0025- . 003	....
Cilia line interval:			
Anterior	. 0025	. 0017	. 003
Middle	. 0031	. 00325	....
Posterior	. 004	. 00325	. 004

The form of the body is sufficiently indicated by the drawings. A characteristic nucleus from individual *A* is seen in figure 197, *b*. Observe the scattered chromatin particles connected by chromatin fibers forming a network. Note also the central achromatic nucleolus. The microchromatin granules and all the achromatic structures except the nucleolus are omitted from the drawing.

Opalina ranarum, United States National Museum No. 16597.

Host.—Rana dalmatina Fitzinger, United States National Museum frog No. 11895, 70 mm. long, from Travnik, Bosnia, 1893; E. Brandes collector; one infection.

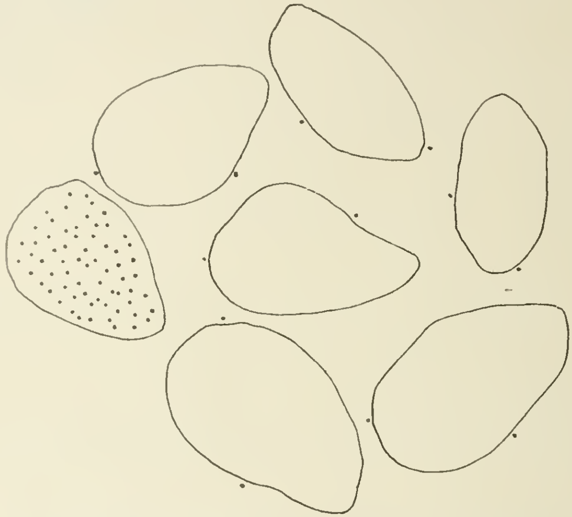


FIG. 198.—OPALINA RANARUM, FROM RANA DALMATINA, × 117 DIAMETERS. ALL THE NUCLEI IN THE CELL ARE SHOWN IN THE LEFT DRAWING.

Measurements of a large individual.—Length of body 0.26 mm.; width of body 0.162 mm.; thickness of body, anterior 0.022 mm., middle 0.019 mm., posterior 0.017 mm.; diameter of nucleus 0.0055 mm. to 0.0062 mm.; cilia line interval, anterior 0.0024 mm., posterior 0.0045 mm.

These seem to be *Opalina ranarum* of the ordinary sort.

OPALINA RANARUM, form TRUNCATA, new forma.

Type.—United States National Museum Cat. No. 16594.

Host.—Rana temporaria, from Würzburg, Germany, collected by M. M. Metcalf in the fall of 1906.

Measurements.—Length of body, 0.34 mm.; width of body, 0.163 mm.; thickness of body, anterior end, thicker side, 0.025 mm., thinner side 0.016 mm.; thickness of body, middle 0.0198 mm., posterior end 0.011 mm.; diameter of nucleus, 0.006 mm. to 0.0075 mm., mean, 0.0069 mm.: diameter of endospherule, 0.002 mm.; cilia line interval, anterior 0.002 mm., middle 0.0035 mm., posterior 0.0052 mm.

This infection, like a number of others of the same sort, is remarkably uniform in the shape of its Opalinas. They are long, are

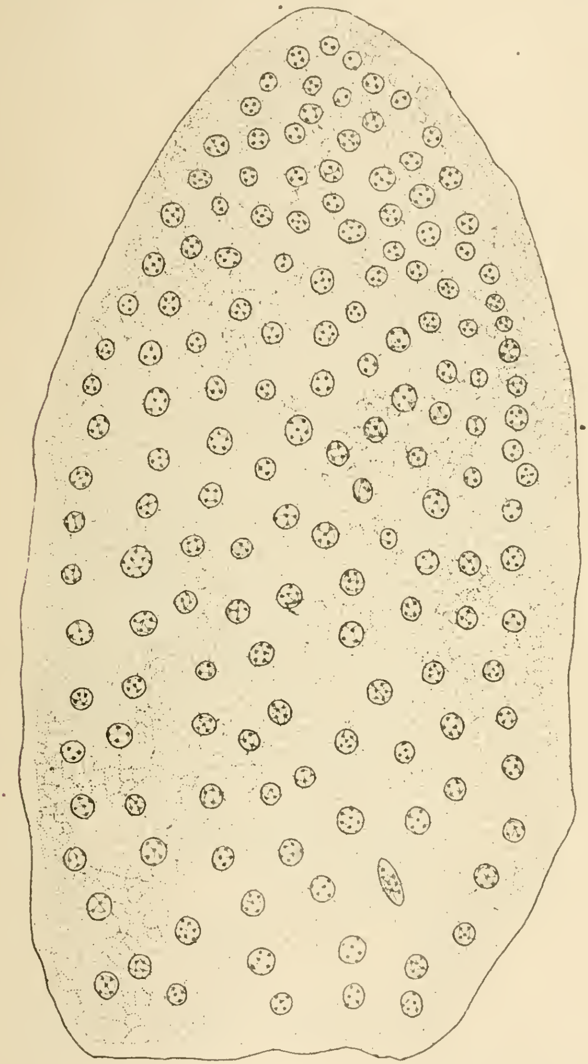


FIG. 199.—OPALINA RANARUM, FORM TRUNCATA, × 460 DIAMETERS.

parallel-sided through the middle portion of the body, are pointed anteriorly and broadly truncated posteriorly, the truncated posterior edge being irregular in contour and very thin. The two small dots outside the contour of the body in figure 199 indicate the limits of the morphological anterior end of the body. This anterior edge is considerably thickened, showing darker in stained specimens. The nuclei and the endosarc spherules are more crowded in this region, and the ectosarc here is thin. The nuclei have their massive chromatin in the form of small, scattered particles.

The differences between these Opalinas and the ordinary, more orbicular *O. ranarum* (figs. 197, 198) seem at first sight to be specific, but in numerous infections from the same species of host one finds these truncated forms along with the orbicular forms and a good many individuals intermediate between the two. It seems therefore, that we have here but a diversity in form within the species. The truncated individuals may be classed as *Opalina ranarum* form *truncata.*

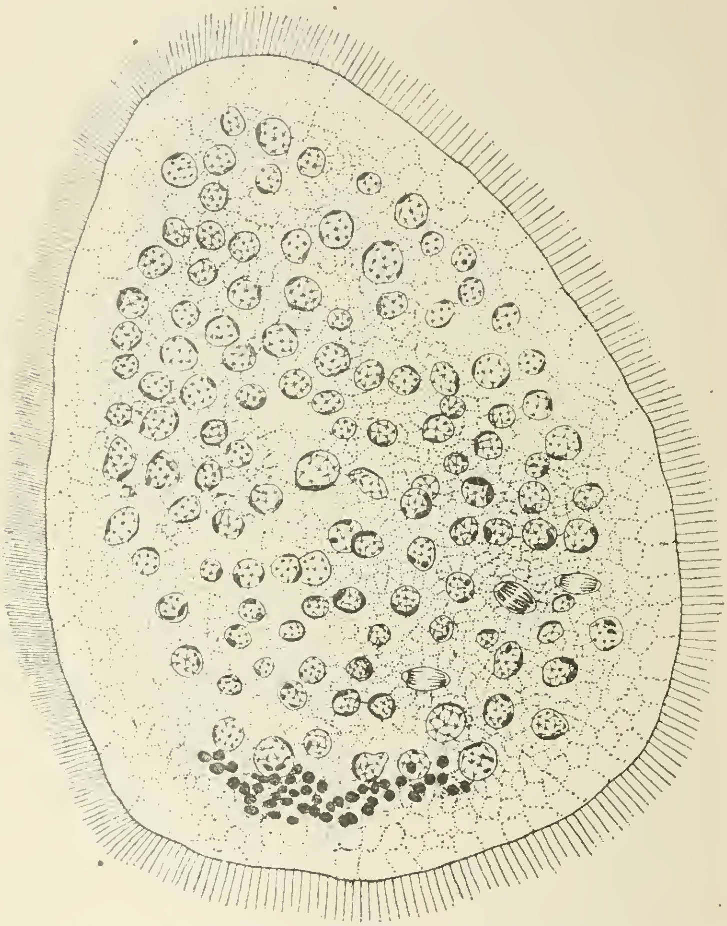


FIG. 200.—OPALINA RANARUM, FORM CINCTOIDEA, × 460 DIAMETERS. NOTE THE CONSIDERABLE CHROMATIN MASSES JUST INSIDE THE NUCLEAR MEMBRANES. FOR THE SAKE OF CLEARNESS THE ENDOSPHERULES ARE OMITTED FROM MOST OF THE FIGURE.

OPALINA RANARUM, form CINCTOIDEA, new forma (figs. 200, 201).

Type.—United States National Museum Cat. No. 16595.

Host.—Rana temporaria, from Würzburg (?), Germany, January 14, 1908.—These were purchased frogs, and the locality is uncertain.

Measurements of an ordinary individual.—Length of body, 0.243 mm.; width of body, 0.185 mm.; thickness of body, anterior 0.02 mm., middle 0.021 mm., posterior 0.02 mm.; diameter of nucleus, 0.0065 mm. to 0.01 mm.; length and width of endospherule, first 0.002 mm. by 0.0011 mm., second 0.0015 mm. by 0.0015 mm.; cilia line interval, anterior 0.0028 mm., posterior 0.0042 mm.; interval between finest longitudinal striae, 0.0009 mm.

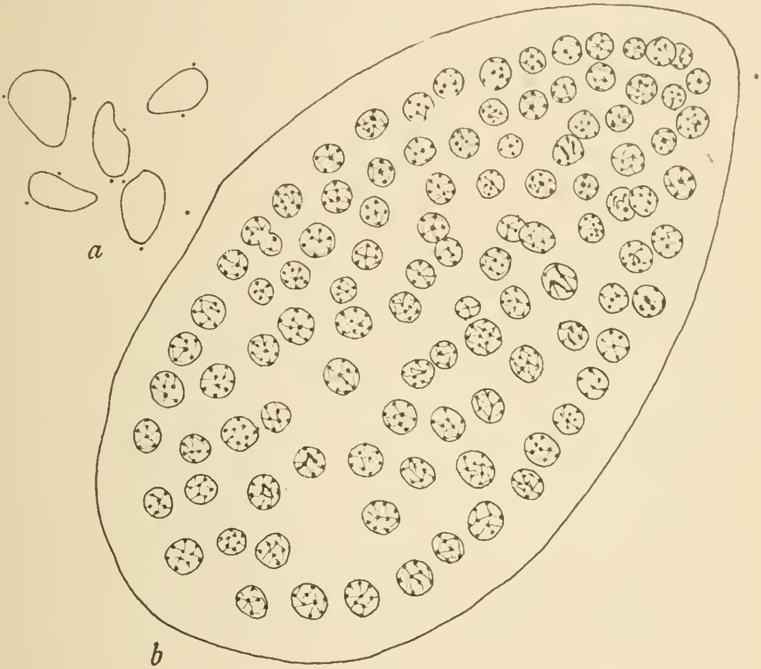


Fig. 201.—Opalina ranarum, form cinctoidea: *a*, a group of animals from one infection, showing range of size and form, × 117 diameters; *b*, an individual of the usual sort, × 460 diameters. Note the finely granular, reticulate condition of the macrochromatin, in contrast to the condition shown in figure 200.

The ordinary individuals in these infections are short and broad, though some are narrower. The posterior end is broadly rounded, or almost truncate. The morphological anterior end is bent to one side, as in all other forms, its distal edge forming the pointed actual anterior end. The ectosarc is very wide around the whole contour of the body, and there is no special thickening of the morphological anterior edge, nor any marked crowding of nuclei or endospherules in this region. The nuclei are very large, some of the largest even slightly surpassing in size the smallest nuclei known in the Protoöpalinas. All of the nuclei show one or more large clumps of massive chromatin under the nuclear membrane. The nuclei seen in mitosis are observed to be shorter and broader than the nuclei from the form *truncata* in a similar stage of mitosis.

One would naturally assign this *Opalina* to a distinct species, but there are found intermediate forms from the same species of host and even in the same infection, grading over into the ordinary *O. ranarum*. It seems necessary to assign them all to the same species, treating them as *O. ranarum* form *cinctoidea*. The name *cinctoidea* is given to this *forma* because of its resemblance to *Opalina cincta* found by Collin in *Bufo bufo* [*B. vulgaris*]. We will discuss Collin's species later. If Collin's species should prove to be merely a form of *O. ranarum*, as is possible, the *cinctoidea* form would be transitional between the form *cincta* and the species type.

OPALINA RANARUM, form PARVIPALMATAE, new forma.

Type.—United States National Museum Cat. No. 16596.

Host.—*Rana temporaria parvipalmata* Sevane, two infections, the type infection from United States National Museum frog No. 38487, 35 mm. long, from Ariége, France, Thomas and Miller collectors; and another similar infection from United States National Museum frog No. 38485, 80 mm. long, a female with eggs, from Parté, France, collected August 31, 1906, by Thomas and Miller.

Measurements of a characteristic individual.—Length of body 0.384 mm.; width of body 0.19 mm.; thickness of body, anterior end, thick edge 0.042 mm., thin edge 0.038 mm., middle of body 0.04 mm., posterior end 0.03 mm., diameter of nucleus 0.0053 mm. to 0.0085 mm.; diameter of endospherule 0.00225 mm. to 0.0025 mm.; cilia line interval, anterior 0.0019 mm., middle 0.00287 mm., posterior 0.0038 mm.

These infections show individuals rather similar to *O. ranarum* form *truncata*, but they are narrowly or broadly rounded behind instead of broadly truncate. In the individual drawn, as in many others, some of the nuclei seem to have disappeared: some large nuclei are in a reticulate condition, others are in mitosis, and there are many smaller nuclei, evidently daughter nuclei in different stages of growth. It seems, therefore, that nuclear division is taking place extensively in these large Opalinas, although one of the hosts was taken the last of August and the other probably was collected at about the same time; and so their Opalinids are not in the midst of the period of rapid division in the spring preceding the sexual phase of the life cycle. The condition of these animals again emphasizes the fact that there are numerous phenomena whose meaning we do not understand, and that there is need of a thoroughgoing study of

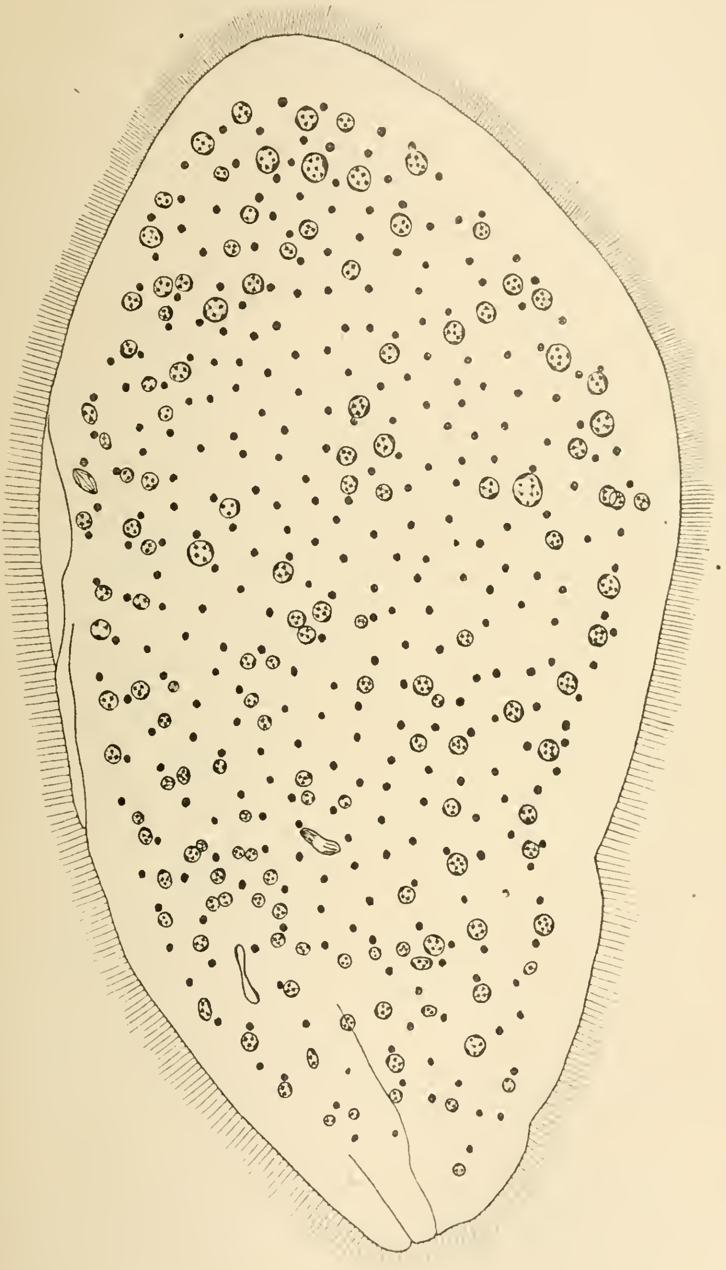


FIG. 202.—OPALINA RANARUM, FORM PARVIPALMATAE, × 460 DIAMETERS.

at least one species of each of the subfamilies *Protoopalinae* and *Opalininae.*

OPALINA RANARUM, form ARVALIS, new forma.

Type.—United States National Museum Cat. No. 16598.

Host.—*Rana arvalis* Nilsson, one abundant infection, from United States National Museum frog No. 37177, 55 mm. long, from Lower Austria, September, 1895; F. Werner collector.

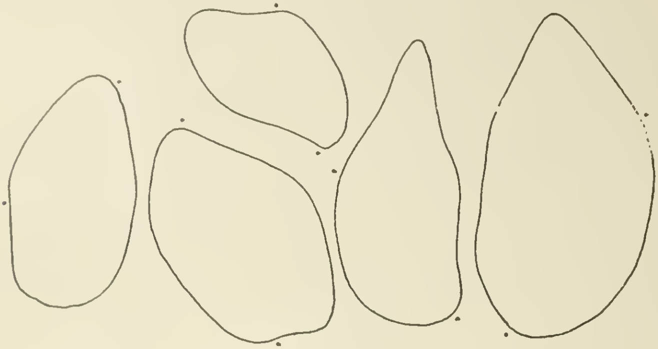


FIG. 203.—OPALINA RANARUM, FORM ARVALIS, × 117 DIAMETERS.

Measurements of an average individual.—Length of body 0.26 mm.; width of body 0.145 mm.; thickness of body, anterior 0.016 mm., middle 0.022 mm., posterior 0.021 mm.; diameter of nucleus 0.005 mm. to 0.0065 mm.; diameter of endospherule 0.002; cilia line interval, anterior 0.0018 mm., posterior 0.0042 mm.

The Opalinas in this infection differ from those from *Rana dalmatina* in being more pointed behind, and in being thinner in front than behind. In the latter regard they differ from all other specimens of *O. ranarum* I have measured, except the form *lata*, next to be described. This difference seems to justify classing them as a distinct *forma*.

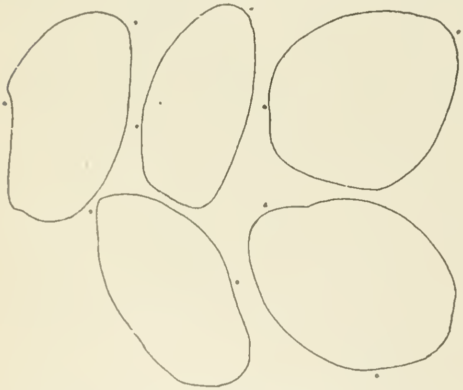


FIG. 204.—OPALINA RANARUM FORM LATA, × 117 DIAMETERS.

OPALINA RANARUM, form LATA, new forma.

Type.—United States National Museum Cat. No. 16603.

Host.—*Bufo viridis* Laurenti, numerous infections from Naples, Italy, April, 1907; M. M. Metcalf collector.

Measurements: A, of a broad individual; B, of a narrower form—

Measurements.	*A*	*B*
	mm.	*mm.*
Length of body	0.188	0.235
Width of body	.2	.137
Thickness of body:		
Anterior	.02	.018
Middle	.021	.0185
Posterior	.021	.016
Diameter of nucleus	0.0065-.0085	0.0062-.008
Diameter of endospherule	.00178	.....
Cilia line interval:		
Anterior	.003	.0031
Posterior	.004	.00337

These infections present Opalinas of two shapes in the same host. The broader forms are often greater in width than in length. Like the form *arvalis* they are thinnest in front, though the difference is but slight. The narrower individuals are truncated behind and irregular in contour, and, like *O. ranarum* form *truncata* are thinnest near this posterior edge. The very broad individuals in these infections are so characteristic that they deserve the *forma* name *lata;* the narrower individuals seem to be the form *truncata.*

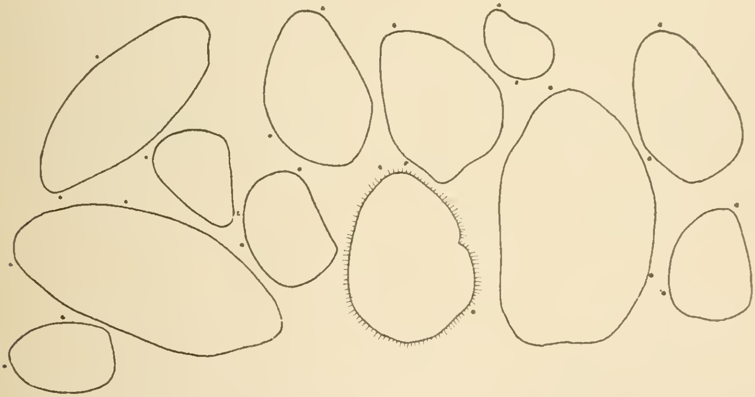


FIG. 205.—OPALINA RANARUM SMITHI, × 117 DIAMETERS. THE CILIATED FIGURE SHOWS AN INDIVIDUAL IN LONGITUDINAL FISSION.

OPALINA RANARUM SMITHI, new subspecies.

Type.—United States National Museum Cat. No. 16607.

Host.—*Bufo smithi* Stejneger, two infections, from Kochi, Japan, May, 1903; H. M. Smith collector. The type infection is from United States National Museum specimen No. 31947, 64 mm. long.

Measurements: A, of a large individual; B, of a long, narrow individual; C, of an average form—

Measurements.	A	B	C
	mm.	*mm.*	*mm.*
Length of body	0.295	0.32	0.0181
Width of body	.175	.142	.113
Thickness of body:			
Anterior	.035		
Middle	.019		
Posterior	.012		
Diameter of nucleus	0.005–.006		
Diameter of endospherule	.0022		
Cilia line interval:			
Anterior	.002		
Posterior	.0048		

This subspecies is almost as varied in shape as *O. ranarum* proper. Like the latter species it is thickest in front, but the difference in thickness between the two ends is much greater in the several individuals measured than it is in *O. ranarum.* The nuclei run smaller than in most *O. ranarum*, but nuclei as small are found in some individuals of *O. ranarum.* The subspecies differs from the species proper in the facts, 1, that its narrower forms differ in shape from both the *truncata* and the *parvipalmatae* forms of the species, 2, that the difference in thickness between anterior and posterior ends is greater, and 3, in having nuclei which are smaller even in the largest individuals.[23] These Japanese infections give quite a different impression from European infections, and one has the feeling that they are really different, but the differences are hard to define. I have not made a statistical study of the several dimensions in the Japanese and European infections, but it is evident that the two groups would center about different means.

OPALINA JAPONICA, new species.

Type.—United States National Museum Cat. No. 16599.

Host.—*Rana japonica* (Guenther), two infections, from Kochi, Shikoku, Japan, May 11: H. M. Smith collector. The type infection is from United States National Museum specimen No. 31907, 48 mm. long.

Measurements of an ordinary individual.—Length of body 0.18 mm.; width of body 0.128 mm.; thickness of body, anterior 0.0105 mm., middle 0.011 mm., posterior 0.007 mm.; length and width of nuclei, first 0.0038 mm. by 0.0038 mm., second 0.004 mm. by 0.004 mm., third 0.0042 mm. by 0.0042 mm., fourth 0.004 mm. by 0.003 mm., fifth 0.0048 mm. by 0.0034 mm., sixth 0.0055 mm. by 0.004 mm., seventh 0.006 mm. by 0.0035 mm.; diameter of endospherule 0.0016 mm. to 0.002 mm.; cilia line interval, anterior 0.0018 mm., posterior 0.0031 mm.

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[23] A paper by Hegner and Hsiang-Fong Wu (1921), of which, through the kindness of Professor Hegner, I have seen a copy in advance of its publication, calls attention to the fact that in young Opalinas with few nuclei the nuclei tend to be larger than in older individuals.

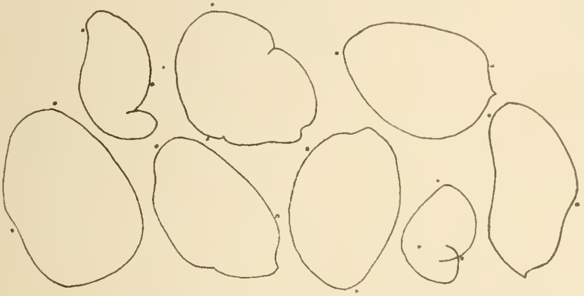

FIG. 206.—OPALINA JAPONICA, FROM RANA JAPONICA, × 117 DIAMETERS.

This eastern *Opalina* differs from *O. ranarum* in having many individuals with the posterior end abruptly sharp pointed. A few have a virguloid form never observed in *O. ranarum.* Also many of the nuclei are more elongated, and some of these do not give indication of being in active motosis. The nuclei, too, run much smaller than in *O. ranarum.* The species seems distinct.

OPALINA JAPONICA (?).

A specimen of this form has been deposited with the United States National Museum as No. 16617.

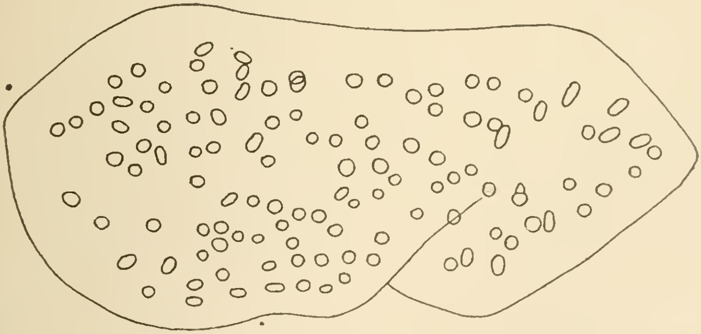

FIG. 207.—OPALINA JAPONICA(?), FROM RANA LIMNOCHARIS, × 460 DIAMETERS.

Host.—Rana limnocharis Wiegmann, one infection, from United States National Museum specimen No. 44158, 35 mm. long, from Mount Gede, Tjibodas, Java, August, 1909; Bryant Expedition, collectors.

Measurements of an ordinary individual.—Length of body 0.2 mm.; width of body, 0.094 mm.; length and width of nuclei, first 0.0045 mm. by 0.003 mm., second 0.0052 by 0.003 mm., third 0.006 mm. by 0.0035 mm.; diameter of endospherule 0.0015 mm.; cilia line interval, anterior 0.0022 mm., posterior 0.0032 mm.

This material is not well preserved, the shape of most individuals being distorted. The general appearance, the measurements, and especially the ellipsoidal nuclei suggest possible identity with *Opalina japonica.*

OPALINA NATALENSIS, new species.

Type.—United States National Museum Cat. No. 16588.

Host.—*Phrynobatrachus natalensis* (Smith), three infections, from Bahr-el-Gebel, Sudan, Africa, March, 1905; F. Werner collector. The type infection is from United States National Museum specimen No. 39477, 20 mm. long.

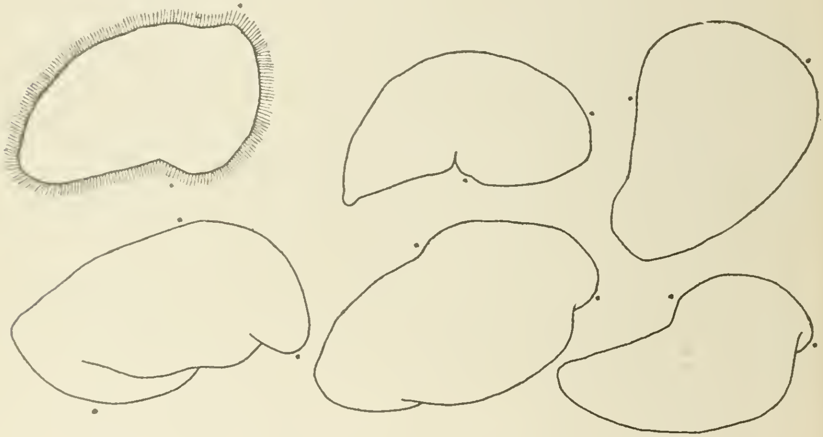

Fig. 208.—Opalina natalensis, × 117 diameters.

Measurements of an ordinary individual.—Length of body 0.3 mm.; width of body 0.15 mm.; thickness of body 0.04 mm.; diameter of nucleus 0.006 mm. to 0.007 mm.; diameter of endospherule 0.002 mm.; cilia line interval, anterior 0.0015 mm., posterior 0.0025 mm.

In form this *Opalina* somewhat resembles *O. japonica*, but its nuclei are larger and are spherical and the animals are in no instance sharp pointed posteriorly. Some of the individuals approach the *Opalinae angustae* in form.

OPALINA CORAOIDEA Bezzenberger (1904).

Hosts.—*Rana cyanophlyctis* Schneider. I have had no material of this species. Bezzenberger reports it from "Asia."

Measurements.—Length of body 0.204 mm.; width of body 0.12 mm.; diameter of nucleus 0.0035 mm.

The characteristic feature is that the sharply pointed posterior end is bent to one side (to the same side toward which the morphological anterior end is bent). *Opalina japonica* shows a similar condition, but much less developed. In this connection compare *Opalina* [*larvarum*], page 244.

OPALINA DRAYTONII, new species.

Type.—United States National Museum Cat. No. 16600.

Host.—*Rana draytonii* Baird and Girard, many living infections from frogs purchased in San Francisco, California (locality of collection doubtful), April, 1913. The type infection is from one of these frogs. Also two infections in United States National Museum specimens from San Pedro Mountain, Lower California (Mexico), and one from another National Museum specimen from San Francisco.

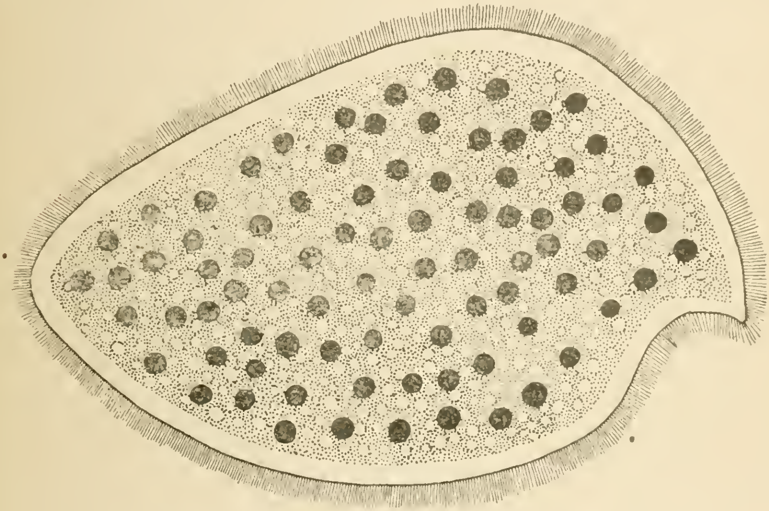

FIG. 209.—OPALINA CORACOIDEA, × 425 DIAMETERS. (AFTER BEZZENBERGER.)

Measurements of an average individual.—Length of body 0.17 mm.; width of body 0.12 mm.; thickness of body, anterior 0.012 mm., middle 0.015 mm., posterior 0.015 mm.; diameter of nucleus 0.004 mm. to 0.0063 mm.; cilia line interval, anterior 0.002 mm., posterior 0.004 mm.

Most individuals from these infections are very broad, others are more slender. The slender forms often, and the broader forms occasionally, show an indication of an abrupt posterior point, which however, is not acute as in *O. japonica*, *O. coracoidea*, and *O.* [*larvarum*], but is itself rounded. The condition is very different from *O. ranarum.* The nuclei, like those of *O. japonica*, run smaller than in *O. ranarum*. The species seems to be distinct, but to resemble *O. japonica*. This resemblance between an eastern Asiatic form and a western American form will be noted in our discussion of geographical distribution as probably indicating a late migration from Siberia to Alaska and south between the mountains and the Pacific ocean. *Opalina draytonii* seems clearly to belong in the group *Opalinae latae* which is an Eastern Hemisphere group.

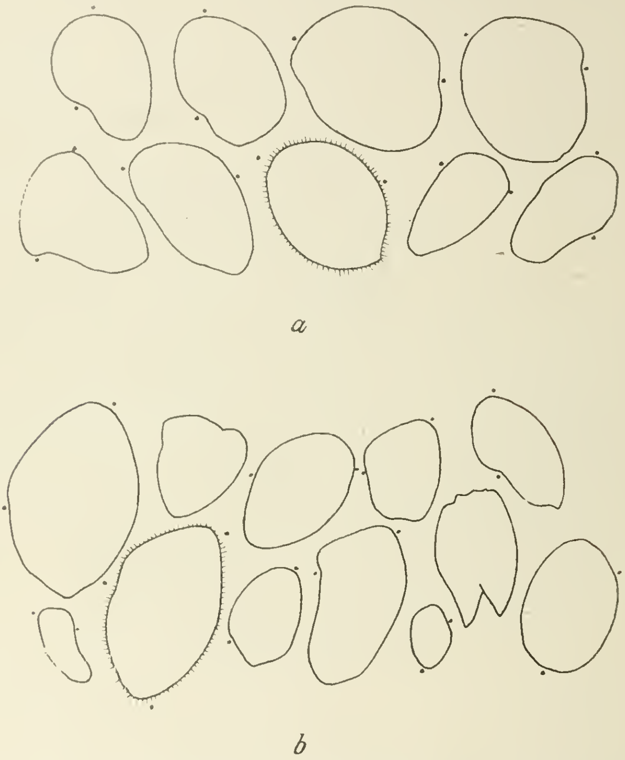

Fig. 210.—Opalina draytonii: *a* and *b*, groups of animals from two different infections, × 117 diameters. In infection *b* were numerous animals in fission and the presence of numerous small cells was probably due to the prevalence of division in this infection.

OPALINA PANAMENSIS, new species.

Type.—United States National Museum Cat. No. 16606.

Host.—*Bufo typhonius* (Linnaeus), two infections, from Bahia Solada, Panama; J. F. Bransford, collector. The type infection is from United States National Museum specimen No. 25176, 32 mm. long.

Measurements of a good-sized individual.—Length of body 0.177 mm.; width of body 0.115 mm.; thickness of body, anterior 0.015 mm., middle 0.015 mm., posterior 0.018 mm.; diameter of nucleus 0.0048 mm. to 0.0056 mm., mean 0.0055 mm.; cilia line interval, anterior 0.00105, posterior 0.0032 mm.

The individuals in these infections are quite diverse in shape. Some are very broad, like *O. ranarum* form *lata* (fig. 211, *c*); others are triangular, being narrow behind, like *O. ranarum* form *parvipalmatae* (fig. 211, *b*); others are narrow and parallel-sided, like *O. ranarum* form *truncata* (fig. 211, *d*). A good many resemble the narrow individuals of *O. japonica*, and, like them, have an abrupt posterior point (fig. 211, *a*). Their spherical nuclei, however, are quite different from the smaller and mostly ellipsoid nuclei of the latter species. Their nearest relatives may well be the Asiatic species *O. japonica* and *O. coracoidea* and the western North American *O. draytonii*. They seem to be western representatives of the *Opalinae latae*, doubtless immigrants.

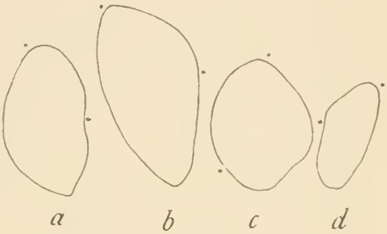

Fig. 211.—Opalina panamensis, × 117 diameters.

OPALINA CAMERUNENSIS, new species.

Type.—United States National Museum Cat. No. 16601.

Host.—*Hylambates rufus* (Reichenow), one very abundant infection, from United States National Museum specimen No. 48850, 75 mm. long, from the Cameroons, west Africa, Barbour collector (?).

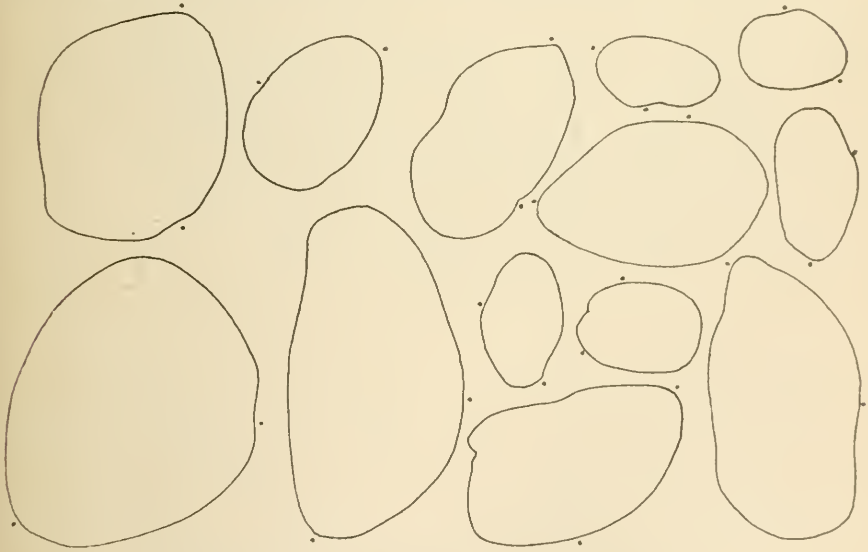

Fig. 212.—Opalina camerunensis, × 117 diameters.

Measurements of a large, broad individual.—Length of body 0.342 mm.; width of body 0.27 mm.; thickness of body, anterior 0.021 mm., middle 0.0345 mm., posterior 0.0275 mm. (a smaller individual has these three measurements of thickness, respectively, 0.016 mm., 0.024 mm., 0.02 mm.); diameter of nucleus 0.004 mm. to 0.0055 mm., mean 0.005 mm.; diameter of endospherule 0.0014 mm.; cilia line interval, anterior 0.00175 mm., posterior 0.00412 mm.; basal granules in the lines of the anterior cilia 800 to the millimeter.

This species is much like *O. ranarum* in shape, except that it is thickest in the middle. Its nuclei run smaller than in *O. ranarum.* Its endospherules are usually small. Its cilia are very numerous anteriorly, the lines of their insertion being close together and the basal granules (cilia) being very close together in these lines. This is a well demarcated species.

OPALINA LATA Bezzenberger (1904).

Host.—*Rana limnocharis* Wiegmann, from "Asia." I have had no material of this species.

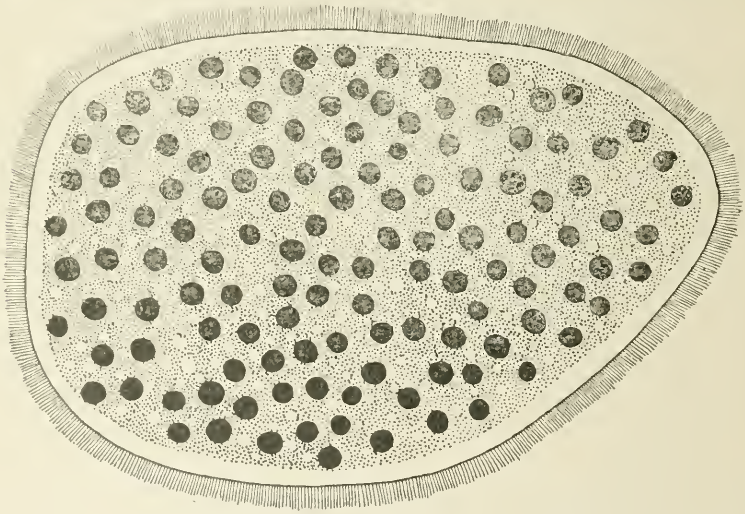

FIG. 213.—OPALINA LATA, × 350 DIAMETERS. (AFTER BEZZENBERGER.)

Measurements.—Length of body 0.3 mm.; width of body 0.18 mm.; thickness of body 0.0126 mm.; diameter of nuclei 0.0049 mm.

The rows of cilia are "extraordinarily close together" and the nuclei are very numerous. This species is *ranarum*-like in form, but has smaller nuclei. It seems also similar to *O. draytonii* from western North America and to *O. camerunensis* from western Africa. The rows of cilia are "extraordinarily close together" in the latter species also, and the cilia are very closely set in the rows, so *O. lata* and *O. camerunensis* agree in being densely ciliated. *O. camerunensis* is nearly twice as thick as *O. lata.*

OPALINA ROTUNDA, new species.

Type.—United States National Museum Cat. No. 16602.

Host.—*Rana erythraea* (Schlegel), one scant infection from United States National Museum frog No. 53518, 28 mm. long, from Ok Yam, Siamese Cambodia, January, 1915; C. Boden Kloss collector.

Measurements of a large, broad individual.—Length of body 0.09 mm.; width of body 0.1 mm.; thickness of body, anterior 0.022 mm., middle 0.026 mm., posterior 0.028 mm.; length and width of nuclei, first 0.005 mm. by 0.005 mm., second 0.0055 mm. by 0.0055 mm., third 0.0055 mm. by 0.0035 mm., fourth 0.0057 mm. by 0.0045 mm., fifth 0.006 mm. by 0.005 mm., sixth 0.0066 mm. by 0.0047 mm., seventh 0.008 mm. by 0.0057 mm.; diameter of endospherule .002 mm.: cilia line interval, anterior 0.00172 mm., middle 0.0025 mm., posterior 0.0025 mm.

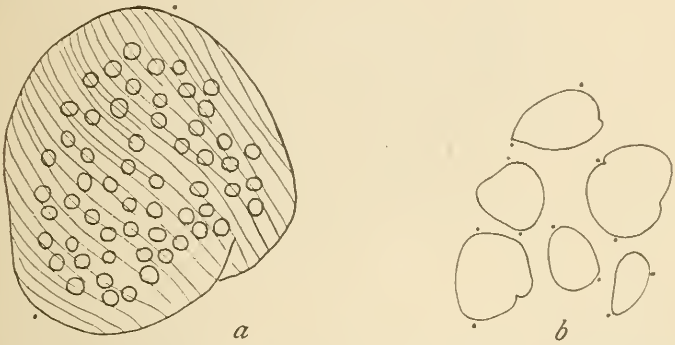

FIG. 214.—OPALINA ROTUNDA: *a* IS MAGNIFIED 460 DIAMETERS, AND ALL THE LINES OF CILIA ON THE UPPER SURFACE ARE SHOWN; *b*, A GROUP OF CELLS, MAGNIFIED 117 DIAMETERS.

This little *Opalina* is usually very broad, but some narrower forms are seen. The body is thinnest in front and thickest behind. Its nuclei are for the most part ellipsoidal, but some are spherical. As the spherical ones are rather small, probably they are daughter nuclei not yet elongated into the typical resting condition. The species seems sharply distinct from any other.

OPALINA CINCTA Collin (1913).

Host.—*Bufo bufo* (Linnaeus) [*Bufo vulgaris* Laurenti], from Viarmes, France. As I have had no material of this species, I quote from Collin's description.

Measurements, taken from Collin's figure.—Length of body 0.141 mm.; width of body 0.104 mm.; diameter of spherical nuclei 0.01 mm., length of anaphase nuclei 0.0194 mm.; width of same 0.0094 mm.

In this orbicular *Opalina* the ectosarc is very wide around the whole contour of the body. The nuclei are very large, larger even than in *O. ranarum* form *cinctoidea*, and almost all are in division. This last is a noteworthy feature in a multinucleated Opalinid. We have seen it in many of the binucleated species, both Protoöpalinas and Zelleriellas, and we have seen numerous Cepedeas and Opalinas with ovoid nuclei, probably resting in a mid-mitotic condition, but in no other multinucleated species known is the picture so clear as in the large nuclei of *O. cincta*. Collin implies that this is characteristic not only of the individual figured, but of the species. It seems an indication that the organisms are not to be regarded as an extreme form of *O. ranarum*, a species with mostly spheroidal nuclei.

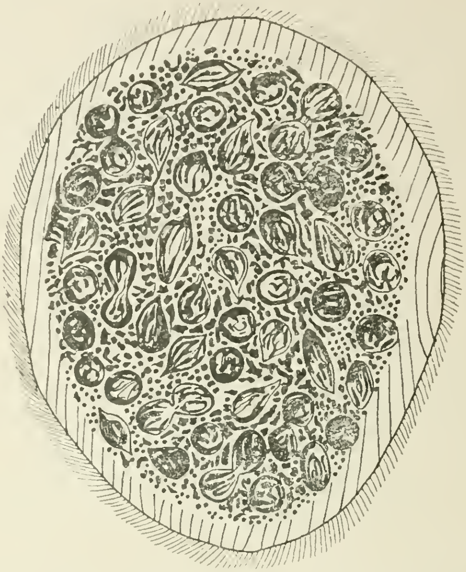

FIG. 215.—OPALINA CINCTA, × 1,500 DIAMETERS. (AFTER COLLIN.) THE ANTERIOR END OF THE BODY IS NOT INDICATED BY COLLIN AND CAN NOT BE DETERMINED WITH CERTAINTY FROM THE FIGURE.

OPALINA RADDEI, new species.

Type.—United States National Museum Cat. No. 16604.

Host.—*Bufo raddei* Strauch, five infections from China. The type infection is from United States National Museum specimen No. 53387, 64 mm. long, from Hei Sui, northeastern Chili, China; A. de C. Sowerby collector.

Measurements: A, of a large individual; B, of a medium-sized individual—

Measurements.	*A*.	*B*.
	mm.	*mm.*
Length of body	0.54	0.355
Width of body	.24	.23
Thickness of body	.042	.036
Diameter of nucleus	0.0025– .004	0.0034– .004
Diameter of endospherule	.0013	.0013
Cilia line interval:		
Anterior		.00185
Posterior		.003

This is a very large *Opalina*, almost the largest among the *Opalinae latae*. Its nuclei are small and it endospherules are minute and very numerous.

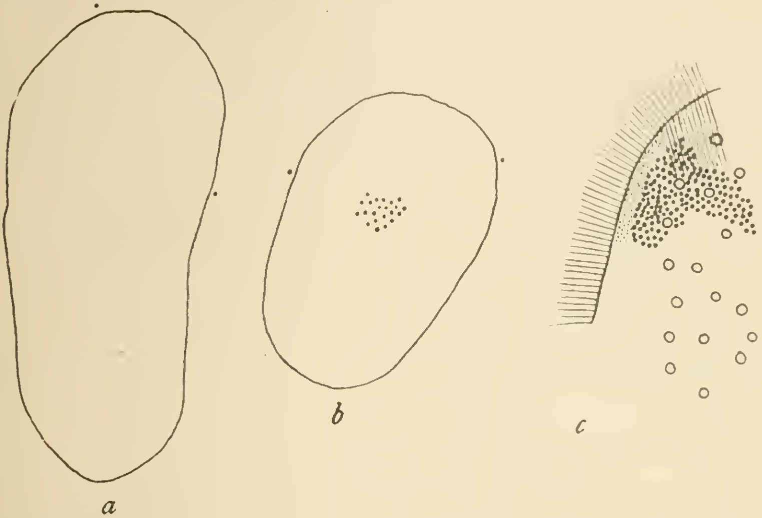

FIG. 216.—OPALINA RADDEI: *a* AND *b*, MAGNIFIED 117 DIAMETERS, *b*, SHOWING A FEW OF THE NUCLEI; *c*, A BIT OF THE BODY MAGNIFIED 460 DIAMETERS, SHOWING CILIA, A PORTION OF THE LINES OF INSERTION OF THE CILIA, ECTOSARC, ENDOSARC, ENDOSPHERULES, NUCLEI.

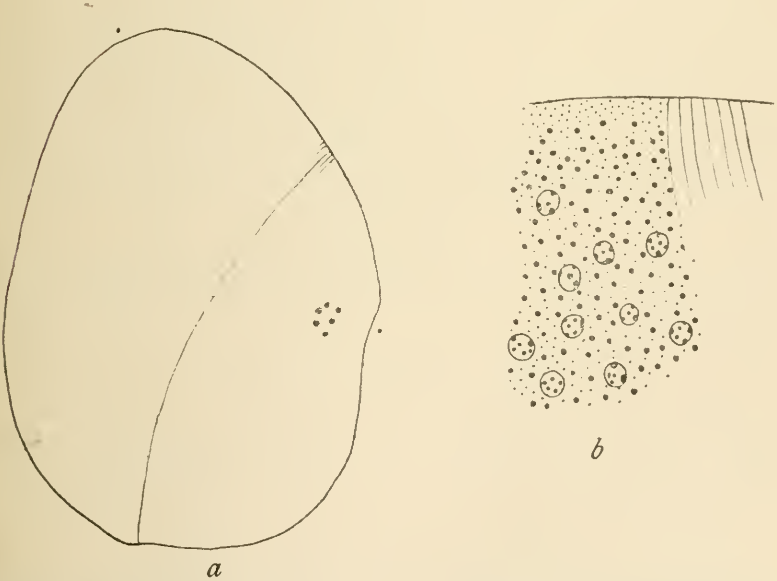

FIG. 217.—OPALINA GIGANTEA: *a*, MAGNIFIED 117 TIMES, SIX NUCLEI BEING SHOWN, ALSO ONE COMPLETE LINE OF INSERTION OF CILIA AND PORTIONS OF FOUR OTHER SUCH LINES; *b*, A BIT OF THE BODY MAGNIFIED 460 DIAMETERS, SHOWING ECTOSARC, ENDOSARC, ENDOSPHERULES, NUCLEI, AND A PORTION OF THE LINES OF INSERTION OF THE CILIA.

OPALINA GIGANTEA, new species.

Type.—United States National Museum Cat. No. 16605.

Host.—*Bufo compactilis* Wiegmann, three abundant infections, from Texas. The type infection is from United States National Museum toad No. 26460, 71 mm. long.

Measurements of a large individual.—Length of body 0.58 mm.; width of body 0.427 mm.; thickness of body, anterior 0.033 mm., middle 0.037 mm., posterior 0.038 mm.; diameter of nuclei 0.008 mm. to 0.009 mm.; diameter of endospherule 0.00094 mm.; cilia line interval, anterior 0.00225 mm., middle 0.003 mm., posterior 0.0045 mm.

This huge *Opalina* is shaped like a broad *O. ranarum*, but often shows clear indication of a short, rounded posterior point. It is thinnest in front and thickest behind. Its nuclei are very large, its endospherules minute and numerous, but not so numerous as those of *O. raddei*. The total number of nuclei in a large individual is about 500.

OPALINA ASIATICA, new species.

Type.—United States National Museum Cat. No. 16608.

Host.—*Bufo bufo asiaticus* (Steindachner), one infection from United States National Museum specimen No. 46617, 102 mm. long, from Shanghai, China, May 26, 1911; D. C. Jansen, collector.

Measurements of a small individual.—Length of body 0.09 mm.; width of body 0.0522 mm.; diameter of nuclei 0.0026 mm. to 0.004 mm.; diameter of endospherule 0.00115 mm.; cilia line interval, anterior 0.00128 mm., posterior 0.00166 mm. An ordinary individual is 0.195 mm. long by 0.086 mm. broad.

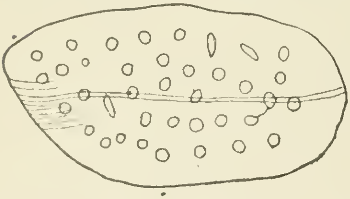

FIG. 218.—OPALINA ASIATICA, × 460 DIAMETERS, SHOWING NUCLEI AND A PORTION OF THE LINES OF INSERTION OF THE CILIA.

This Opalina has very small nuclei and minute endospherules. In shape it approaches *O. ranarum* from *truncata*. It seems clearly a distinct species.

OPALINA [BUFOXENA], new species (?).

A specimen of this form has been deposited with the United States National Museum as Cat. No. 16609.

Host.—*Bufo bufo asiaticus* (Steindachner), one infection, from United States National Museum specimen No. 52355, 63 mm. long, from southern Manchuria; A. de C. Sowerby, collector.

Measurements of an ordinary individual.—Length of body 0.18 mm.; width of body 0.098 mm.; thickness of body 0.028 mm.; length and width of nuclei, first 0.0056 mm. by 0.004 mm., second 0.0058 mm. by 0.0045 mm., third 0.0075 mm. by 0.005 mm., fourth 0.0075 mm. by 0.0055 m.; length of endospherule 0.0014 mm.; width of endospherule 0.0011 mm.; cilia line interval, anterior 0.00125 mm., posterior not much greater, but not clear enough to measure.

This *Opalina* is one of three forms found in this oriental toad. Its chief diagnostic characters are, 1, the ellipsoidal nuclei (improperly drawn in this figure); 2, the small, elongated endospherules; 3, the rather long and very crowded cilia; 4, the slight difference in density of ciliation between the anterior and the posterior ends of the body.

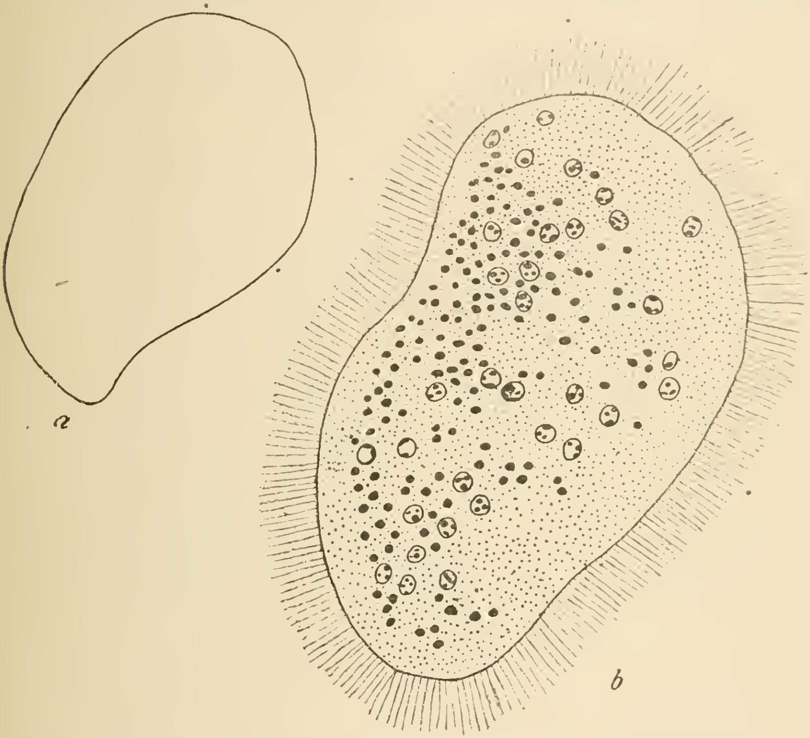

Fig. 219.—Opalina [bufoxena], × 460 diameters. Note in *b* the irregular distribution of the few nuclei and of the few endospherules. All the nuclei and endospherules observed are drawn. The individual is apparently abnormal.

In figure 219, *b*, is shown an individual many of whose nuclei seem to have degenerated. Smaller nucleuslike bodies are present, but are not well stained. The large nuclei present are not spheroidal as drawn, but are shrunken and of irregular contour. The irregular distribution of the few endospherules is an indication that the individual is abnormal.

It seems possible that this may be but a form of *O. asiatica.* It is found in the same host and its dense ciliation resembles that of the latter species. In both the endospherules are small. The chief difference is in the normal nuclei, these being very small and spherical in *O. asiatica*, while they are very much larger and ellipsoidal in *O.* [*bufoxena*]. Until we understand somewhat more definitely the nuclear phenomena throughout the whole life cycle, we must leave in doubt the connection between these two forms of *Opalina* from *Bufo bufo asiaticus.*

OPALINA, species (?).

Host.—*Hyla septentrionalis* Boulenger, United States National Museum No. 51867, Los Hermanos Mountains, Cuba, May 31, 1914, P. Bartsch, collector.

This is a narrow species. The material is unsatisfactory for description. No specimens have been deposited with the United States National Museum.

OPALINA, species (?).

A specimen of this form has been deposited with the United States National Museum as No. 16618.

Host.—*Rana mascareniensis* Duméril and Bibron, one infection, from United States National Museum specimen No. 33112, from the Gold Coast, Africa, A. Guenther, collector (?).

This material is not well enough preserved for study. The Opalinas are multinucleated, broad and flat and seemingly belong among the *ranarum*-like species. They are small, 0.18 mm. long by 0.136 mm. wide. In the same host are very numerous *Nyctotherus*. No specimen of this form has been deposited with the National Museum.

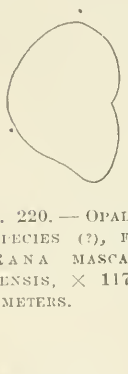

Fig. 220.—Opalina, species (?), from Rana mascareniensis, × 117 diameters.

OPALINA [LARVARUM], new species (?).

A specimen of this form has been deposited with the United States National Museum as Cat. No. 16629.

Host.—Tadpoles of *Rana clamitans* Latreille (?), from Chester, Nova Scotia, June 10, 1920, Dr. Charles E. Simon, collector.

Measurements: A of an ordinary individual; B of a broad individual; C of a slender individual.—

	A	*B*	*C*
	mm.	*mm.*	*mm.*
Length of body	0.073	0.0717	0.075
Width of body	.0545	.0684	.0455
Length of posterior process	.005	.0056	....
Diameter of nucleus	0.0065–.0075	....	....
Length and width of anaphase nucleus	.0175×.005	....	....
Cilia line interval:			
Anterior	.00315	....	....
Middle	.0058	....	....

Adult *Rana clamitans* have yielded no Opalinids, though 33 specimens from 8 localities have been examined. Professor Hegner, of the Johns Hopkins University School of Hygiene and Public Health, sends me a slide, a smear preparation from a tadpole of what seemed to be *Rana clamitans*, containing numerous Opalinas all of the sort shown in figure 221. These are distinct from any *Opalina* as yet seen. They are very broad, almost circular in form, but each individual bears posteriorly a short, curved, sharp-pointed protuberance. [See *Opalina coracoidea*, p. 234.] The shape of the body seems to demarcate sharply this *Opalina* from any other species, but, as the young individuals of many species have never been observed, it is unsafe to name the present species definitely from the very young specimens which are the only ones available for study. The sharp, curved, posterior point suggests that this *Opalina* may be one of the western hemisphere narrow species, even though its body in this young stage is very broad. Young *Opalina hylaxena*, from the tadpoles of *Hyla versicolor*, are almost as broad as the specimens of *O.* [*larvarum*], though the adults of *O. hylaxena* are elongated and slender. It is highly probable that this *Opalina* from the Nova Scotian tadpoles is a distinct species, but it is best to name it only tentatively. The identification of the species of the host is somewhat uncertain.

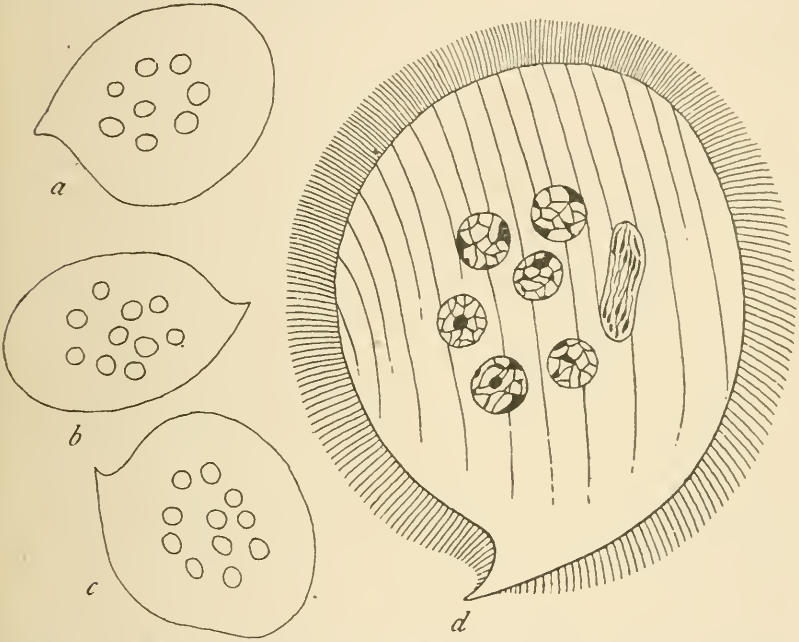

FIG. 221.—OPALINA [LARVARUM]: *a*, *b*, AND *c*, MAGNIFIED 460 DIAMETERS; *d*, MAGNIFIED 1,000 DIAMETERS.

It is of interest if the tadpoles of this frog are infected while the adults are not known to carry Opalinids. Somewhat similar relations obtain in *Hyla versicolor*, for, though only a small percentage of the adults of this species harbor *Opalina*, the many tadpoles studied have all been abundantly infected with *O. hylaxena.* Possibly the tadpoles of *Rana catesbeiana* and *Rana grylio* may carry Opalinids, though I have examined many tadpoles of the former species without ever finding these parasites. The adults of either species of bullfrog have never been reported as infected, excepting specimens of the former species introduced into Hawaii and from there imported into California. Native American specimens of these species have proven barren.

There are intestinal protozoa, other than the Opalinidae, which, while not reported from adult Anura, are well known from the larvae, as for instance *Giardia.* Professor Kofoid, in conversation, suggested that the change from the vegetarian diet of the tadpoles to the carnivorous diet of the adults may account for the disappearance of the *Giardia* at the time of the metamorphosis of the host. There are changes in the intestinal tract itself at this time, changes involving reconstruction of the tissues of the host, and probably accompanied by a period of fasting. If this change of diet and the accompanying tissue changes involving abstention from food influence unfavorably the Opalinids in some species of hosts, they are but little, if at all, hurtful in other species, for Opalinids are abundant in most adult Anura. It is perhaps worth noting that the green frog and the two species of bullfrog mentioned are our largest American frogs and are the only North American frogs not known to harbor Opalinids when adult. On the other hand adult Bufos of several species of even larger size are abundantly infected with Opalinids.

The tadpoles of *Rana catesbeiana, R. grylio, and R. clamitans* live over one or even two winters in the tadpole state. It seems probable that the tadpoles of a previous season may directly infect the tadpoles of a new season, so that there is not need of their passing through the difficult (?) metamorphosis perod of their host. Brumpt (1915) has shown that among European Anura the Opalinids in the older tadpoles regularly go through a period of rapid division followed by encystment, just as they do in the adult Anura in the spring, and that younger tadpoles become infected from these ejected cysts. Any species of Anura whose tadpoles live over winter might then readily show Opalinids in the tadpoles and not in the adults.

THE GENERA AND THE SUBFAMILIES OF THE OPALINIDAE.

After the foregoing description of the species in the family, the characters of the several genera are clear. *Protoopalina* includes those species which are circular in cross section, or at least not greatly flattened. They have two large nuclei, 0.01 mm. or more in the longest diameter, except that one species *P. primordialis*, is described as having a single nucleus characteristically in mitosis. In just what stage of mitosis Awerinzew, the discoverer, does not say. There are also two species with more than two nuclei; one, *P. quadrinucleata* regularly has four nuclei, and the other, *P. axonucleta*, may have as many as eight or more nuclei, usually in pairs. The nuclei in this genus have few (from 4 to 10) macrochromosomes; the number of microchromosomes has not been determined except for two species, but they are but few, apparently no more numerous than are the macrochromosomes. In mitosis both the macrochromosomes and the microchromosomes are equally divided, one of each pair going to each daughter nucleus.

Zelleriella includes the much flattened binucleated species. These also have large nuclei, about 0.01 mm. or more in greatest diameter, and they have few macrochromosomes, 4 to 10, so far as observed. The microchromosomes, in the one species studied as to this point, are equal in number to the macrochromosomes, and in some other species the numbers of the two sets of chromosomes are about equal, if not exactly so. In mitosis both the macrochromosomes and the microchromosomes are equally divided, one of each pair going to each daughter nucleus. These two genera together constitute the subfamily *Protoopalininae*, which includes all the binucleated species both cylindrical and flat.

The subfamily *Opalininae* includes the multinucleated species, with usually small nuclei. The number of macrochromosomes, in those species in which it has been determined, is 12 or more. The microchromosomes are present in about the same number, but have not been well studied. In the mitosis, at least of some species, some of the macrochromosomes may not divide equally, or even may not divide at all. Some of the nuclei of these multinucleated forms may thus become distorted in their chromatin balance and this may account for the presence of occasional degenerating nuclei. No similar irregularity of division of the macrochromosomes has been observed in any *Protoopalina* or *Zelleriella*. The subfamily *Opalininae* includes two genera: *Cepedea*, including the species which in cross section are circular, or nearly so, or at least are not uniformly flattened throughout the body; some species are considerably flattened, especially anteriorly; and *Opalina*, including the uniformly flattened species, among which is *Opalina ranarum*, the type species of the family. A division of the genus *Opalina* into *Opalinae angustae*, more or less slender species with the posterior end narrowed or pointed (*obtrigona*-like or *virgula*-like forms), and *Opalinae latae*, broader species with the posterior end usually broadly rounded (*ranarum*-like forms), is emphasized by the fact that the slender species are American, while the broad species occur in the eastern hemisphere. These subgeneric groups are not sharply demarcated, for some of the western hemisphere species have broad individuals and some of the eastern hemisphere species show narrow forms. It is not possible to be quite sure, in some few instances, to which subgeneric group a species belongs. The American group has sent one known representative into Asia and Europe, and the *Opalinae latae* of eastern Asia have sent a few representatives into western America. Further reference to these two groups of species of *Opalina* will be made in discussing geographical distribution (sec. 7).

Only the four genera, *Protoopalina*, *Zelleriella*, *Cepedea*, and *Opalina*, are to be included in the family Opalinidae. Other genera of astomatous Ciliata, which have often been included in the family, are fundamentally different in nuclear character. The relationships of the Opalinidae to one another will be discussed in section 5, and the relationships of the family Opalinidae to other Protozoa will be discussed in section 6.

Subgeneric groups among the Opalinids are discussed in section 7 of this paper in connection with problems of distribution. In that discussion we will find reason for believing that *Protoopalina* and *Cepedea* are old genera and that *Zelleriella* and *Opalina* were more recently evolved, and we will note that the two older genera are divisible into subgeneric groups of species, while *Zelleriella* is a very compact genus with no such subgeneric group, and that *Opalina* shows only the division in *Opalinae latae* and *Opalinae angustae*. The two older genera have developed a more divergent speciation.

It is worthy of note that the tendency to become flattened has appeared at least twice in the Opalinidae, each subfamily containing a more archaic cylindrical genus from which has been derived a flattened genus. As already noted, it is possible that flat Opalinas have arisen twice or even oftener from Capedeas.

4. THE NUCLEAR CONDITIONS IN THE OPALINIDAE.

The Opalinidae show several features of nuclear structure and behavior that are of much interest:

1. Alone among the Ciliata, they have two or more nuclei which are all *alike*, all other Ciliates having two nuclei differentiated to form, one a metabolic meganucleus, the other a reproductive micronucleus; temporarily in some Euciliates, the macronucleus may be divided into numerous portions and at certain stages in the life cycle there may be several micronuclei.

2. Each nucleus of the Opalinids has both metabolic and reproductive chromatin fully developed and functional, the metabolic

chromatin in the form usually of large, flat masses lying just beneath the nuclear membrane, the reproductive chromatin in the form of much smaller spheroidal granules.

3. In the binucleated genera, *Protoopalina* and *Zelleriella*, both sorts of chromatin are, during mitosis, in the form of definite chromosomes, the macrochromosomes (metabolic) being ribbon-shaped, and the microchromosomes (reproductive) being linear aggregates of granules.

4. In some species, perhaps in all, the number of macrochromosomes equals the number of microchromosomes.

5. There are, in some species at least, constant differences of form and size between the macrochromosomes, allowing us to recognize individuality in these chromosomes. Similarly the microchromosomes differ among themselves in the number of granules they contain, some being long strings of numerous granules, others being very much shorter and having many fewer granules, others, still, being intermediate. The constancy of these individual microchromosome characters has not yet been determined by comparison of different nuclei.

6. There are no centrosomes in the nuclei or in the cytoplasm, in the Opalinidae.

7. The mitotic spindle fibers are of three (?) sorts: *a*. Chromatic threads which are, as it were, filose pseudopodia from the macrochromosomes. During the granulated condition of the nuclei these chromatin fibers branch and form a coarse reticulum. The macrochromosome fibers and their branches are peripheral, just beneath the nuclear membrane. Each macrochromosome is attached at each end by such a chromatin fiber, more or less branched according to the stage of mitosis, to the corresponding pole of the nuclear membrane. Probably these macrochromosome fibers have a linin core. The macrochromosomes themselves apparently do have. *b*. Chromatin threads, of similar size, lying a little more centrally, which connect each end of each microchromosome with the corresponding pole of the nuclear membrane. The condition of these microchromosome fibers has not yet been thoroughly studied throughout the whole mitotic cycle. *c*. The achromatic portion of the mitotic spindle, lying in the axis of the nucleus, seems somewhat fibrillar, but the apparent delicate fibrillae may be but emphasized longitudinal films of the achromatic alveolar substance.

8. The fact that each chromosome is bound by each end to the corresponding pole of the nuclear membrane, and that the connection is not lost even in the reticulate phase of the mitotic cycle, makes these nuclei particularly favorable for the study of certain cytological problems.

9. It is also of interest to note that there is, in the *Protoopalininae* at least, no rotation of the nuclei within the body, the major axis of each nucleus maintaining a constant relation to the major axis of the body, except for a slight swaying, of not more than twenty degrees, at the time of lingitudinal fission. This constancy of orientation both of nuclei and of chromosomes within the nucleus opens up interesting possibilities of cytological study.

10. The Opalinidae, especially the *Protoopalininae*, are unique among organisms in that, in the case of many species, their nuclei are not customarily found in a reticulate condition, but rather come to "rest" in some other phase of the mitotic cycle, the particular mid-mitotic phase chosen being characteristic of the species. Species whose nuclei thus regularly "rest" in some mid-mitotic condition are very numerous in the genus *Protoopalina*, and are less numerous in the genus *Zelleriella*. Among the multinucleate forms, the *Opalininae*, species with nuclei resting in a mid-mitotic condition are fairly numerous in the genus *Cepedea*, but are fewer in the genus *Opalina*. The ellipsoidal form of the nuclei in some species of *Opalininae* is a readily observed indication of probable mid-mitotic condition.

It is this peculiar habit, of having their nuclei come to rest before having completed their mitosis, which gives probably the chief key to the speciation in the family. Furthermore it seems to tell us much about the origin of the Ciliata, whose higher members, the Euciliata, have perhaps the most peculiar nuclear conditions known among organisms. The Opalinidae help us to interpret the nuclear phenomena in the Euciliata, and they enable us to judge something as to the ancestral conditions and the evolution of this unique group.

In the description of the species of the genus *Protoopalina* we began with Awerinzew's uninucleated *P. primordialis* and passed to the eight(±)-nucleated *P. axonucleata* through a series of species with nuclei in intermediate conditions. Let us rapidly review the several steps in this ascending series, turning as we do so to the figures of the several species named.

Protoopalina primordialis, nuclear condition not adequately described, but said to have but one nucleus, in dumb-bell condition, that is, in the midst of mitosis.

P. diplocarya (fig. 11, p. 33), a species with two spherical nuclei, still united, however, by a thread; that is, the two daughter nuclei are not yet completely separated. Each nucleus is in a very early metaphase, the four chromosomes being still distinct.

P. papuensis (fig. 12, p. 35), a species with two pear-shaped nuclei united by a thread; that is, the two daughter nuclei have not completely separated. Each nucleus is usually in a skein condition.

P. caudata (fig. 19, p. 42), with two nuclei, usually pear-shaped (metaphase condition), but sometimes almost spherical (reticulate condition). When pear-shaped the nuclei are often still connected by a thread.

P. macrocaudata (fig. 25, p. 50), with two nuclei connected by a thread. Each nucleus, in the characteristic condition, is in a later mitotic phase than is the ordinary resting nucleus of *P. caudata*.

P. intestinalis (fig. 27, p. 52), with two nuclei frequently not connected by a thread and usually in a late metaphase, almost a reticulate condition.

P. tenuis (fig. 34, p. 59), with two distinct nuclei, each being usually considerably elongated preparatory to the next mitosis. The internal structure of the characteristic nucleus of this species shows a reticulate or even a prophase condition.

P. regularis (fig. 41, p. 70), with two elongated nuclei, still connected by a thread, usually each in an early anaphase of mitosis.

P. scaphiopodos (fig. 46, p. 76), with two distinct dumb-bell-shaped nuclei, each regularly in a very early metaphase.

P. mexicana (fig. 49, p. 79), with two distinct dumb-bell-shaped nuclei, each usually in a somewhat later metaphase.

P. formosae (fig. 50, p. 81), with two distinct nuclei, each almost completely divided into two; the four pear-shaped daughter nuclei are still connected in pairs by slender threads. Each is an early telophase.

P. quadrinucleata (fig. 51, p. 82), with four distinct nuclei, each elongated and in an early stage of the next mitosis, with distinct chromosomes, not, however, as yet arranged upon a mitotic spindle.

P. axonucleata (fig. 52, p. 83), with usually from 6 to 10 nuclei in pairs. The nuclei may, in different individuals, be in different stages of mitosis, and in a single individual there may be some difference in the mitotic phases of the different pairs of nuclei, one pair being, say, in a dumb-bell condition, not yet divided, another showing the daughter nuclei still connected merely by a thread, while a third pair may show the daughter nuclei disconnected.

It seems that the series of conditions here noted may correspond to the series of evolutionary stages by which originally uninucleate forms acquired the binucleate condition and ultimately passed on to the multinucleate condition.

Among the Zelleriellas we find none that are regularly uninucleate even with a dumb-bell nucleus. Of course, after fission one of the daughter cells may be for a time uninucleate. Nor do we find as full a series of characteristic midmitotic resting conditions in the several species of this genus. One naturally judges that the flattening of the body, which produced the Zelleriellas, took place after the possession of two nuclei became characteristic of the

Opalinid stock. This is in keeping with the conclusion, reached in our discussion of the data from geographical distribution in section 7, that Zelleriella is a comparatively modern genus. None of the Zelleriellas known are multinucleate, as are *Protoöpalina quadrinucleata* and *P. axonucleata*.

Among the *Opalininae*, both the Cepedeas and the Opalinas, we see species with spherical, resting nuclei in the granular reticulate condition, and other species with ellipsoidal nuclei, but we find no such series of characteristic midmitotic resting conditions in the several species as the *Protoopalininae* present. The *Opalininae*, as a whole, being multinucleated, may be taken as showing the last major term in the series of nuclear conditions in the family.

There is evidenced in the family as a whole, when its species are thus comparatively studied, a strange tendency hindering the prompt completion of nuclear division, and at the same time delaying division of the body. This inhibiting tendency produces results in some respects unique among organisms. In no other group of animals or plants are there known nuclei which, having once entered upon mitosis, regularly come to rest without promptly completing the mitotic process. It is remarkable, in the light of phenomena in other groups, that there should be any such prolonged inhibition of the completion of mitosis. It is still more remarkable to find, as we do, especially among the Protoöpalinas, a series of species whose delayed mitoses are interrupted at different, specifically characteristic phases of the mitotic cycle, so that in some species the resting nuclei are in an early prophase, in others in a late prophase, in others in about the equatorial plate phase, in others in an early anaphase, in others in a late anaphase, in others in an early metaphase, in others in a late metaphase, while still others have their nuclei resting in a reticulate condition similar to the usual resting condition of the nuclei in other organisms.

Of course, in any species of *Protoopalina*, or of the other genera of Opalinidae, we find all stages of mitosis, but in most species there is a resting condition, mid-mitotic or otherwise, as definite and evident as the resting condition of the nuclei in other organisms. In a few species, as for instance in *Zelleriella paludicolae*, either there is greater latitude in the condition in which the nuclei come to rest, or some of my material has been killed at a time when division was very active, so that nuclei in all stages of mitosis are found in abundance. The latter seems the wholly probable explanation, for not all my infections of *Z. paludicolae* show this exterme diversity in mitotic condition.

The partial inhibition of nuclear division in the Opalinids is paralleled by a partial inhibition of the division of the body. In the binucleated species, and still more in the multinucleated species, after the nucleus has divided, the division of the body is still delayed, this delay in the division of the body being the thing that causes the binucleated and multinucleated conditions. In *Protoopalina* and *Zelleriella*, which are characteristically binucleated, the nuclei have already divided into two, but the corresponding division of the body does not occur. It does, however, occur in time. When the two nuclei again enter upon mitosis, the delayed division of the body takes place, so that, except in two species, not more than two distinct nuclei are found in the one body.

Different species differ as to the exact mitotic condition in which the nuclei will be found at the time when division of the body occurs. In some species, as for example *P. saturnalis* (fig. 37, p. 64) or *P. stevensoni* (fig. 26, p. 51), the two nuclei are ellipsoidal at the time of division of the body. In *P. saturnalis* each nucleus is at this time in a diffuse reticulate condition. In other species, as *P. caudata* (fig. 19, p. 42) and *P. intestinalis* (fig. 27, p. 52), each nucleus is in an elongated ellipsoidal anaphase when the body divides. In *P. regularis* (fig. 41, p. 70) each nucleus is in a slender spindle-shaped anaphase. In *P. pelobatidis* (fig. 16, p. 39) each nucleus is in a dumb-bell-shaped anaphase; so also in *P. bufonis* (fig. 45, p. 74). In *P. scaphiopodos* (fig. 46, p. 76) and *P. mitotica* (fig. 48, p. 78) both nuclei are in a dumb-bell-shaped early metaphase before the body divides. In *P. mexicana* (fig. 49, p. 79) division of the body does not occur until each nucleus has reached a dumb bell-shaped late metaphase condition. In *P. formosae* (fig. 50, p. 81) the fission occurs only after each of the two nuclei has given rise to two almost distinct daughter nuclei connected only by a slender thread of considerable length. In *P. quadrinucleata* (fig. 51, p. 82) the two nuclei have completely divided into four, and division of the body does not take place until each of these four nuclei has entered upon the next mitosis. In *P. axonucleata* (figs. 52 and 53, pp. 83 and 84) as many as 10, possibly in some cases more, nuclei may be present before division of the body occurs.

A somewhat similar, but less extensive series could be given for the species of *Zelleriella*. *Z. dendrobatidis* (figs. 63 and 64, p. 96) may divide while the two nuclei are spherical, or only a little elongated, while, at the other extreme, *Z.* [*engystomopsis*] (fig. 62, p. 95) does not divide until each nucleus has completely divided into two, so that there are four distinct nuclei in the body. Between these two extremes are numerous forms, as *Z. paludicolae* (fig. 70, p. 103) and *Z. darwinii* (fig. 74, p. 108), which divide while the two nuclei are spindle shaped, *Z. atelopyxena* (fig. 59, p. 92) and *Z. hylaxena* (fig. 78, p. 112), which divide while the two nuclei are dumb-bell-shaped, and *Z. antilliensis* (fig. 84, p. 118), which divides while the two daughter nuclei of each pair are connected merely by a thread.

In the multinucleate forms, the *Opalininae*, there is still further inhibition of the division of the body, until, in the most extreme species, we find individuals with several hundred nuclei. In *Cepedea segmentata* (fig. 140, p. 173) we see the inhibition of the division of the body carried to the greatest extreme, many fissions having begun but remaining uncompleted, so that the body shows a series of segments.

But however much the division of the body may be hindered and postponed, in the end each suppressed division succeeds in taking place; for, as a result of the rapidly occurring divisions, preceding the sexual phenomena, there are produced ultimately uninucleate gametes. The body divisions are not lost, they are merely temporarily suppressed. Even the first body division, whose suppression gives the Protoöpalinas and the Zelleriellas their binucleated character, reappears in the end, for the gametes of these genera are regularly uninucleate.

It is very interesting to find in the Euciliata indication of similar suppression, but not absolute obliteration, of that division of the body which, if it occurred, would make the Euciliates uninucleate rather than binucleate forms. In the year 1912 Dr. Peebles showed that when the posterior third or quarter, say, of a *Paramecium* is cut off, the anterior moiety does not regenerate until a division occurs along the line in which the next regular fission would have taken place if the animal had not been mutilated.[24] There is in *Paramecium* a potential division plane already physiologically laid down, and under the stimulus of mutilation the division itself appears. This seems to be the suppressed division whose omission under undisturbed conditions makes *Paramecium* a binucleated instead of a uninucleated organism. In the Euciliates, under normal conditions, there is complete suppression of one division of the body, and the animals have two or more nuclei in all phases of the life history. In the Opalinidae, on the other hand, all suppressed divisions finally appear, and the organisms during the sexual phase of the life cycle become uninucleate. The Opalinidae are pseudopleurinucleate forms, the Euciliata, on the other hand, are permanently binucleated.

Connected with this difference between true and false binucleation in the Euciliates and Opalinids are the relations which obtain in the two groups between the nuclei and the planes of division in fission. In the Euciliata both macronucleus and micronucleus elongate and become dumb-bell shaped at the time of fission, and the division plane passes between and separates the two daughter nuclei of each pair, large and small, so that each daughter cell receives one-half of each of the two former nuclei. In the Opalinidae, on the contrary, the plane of division, both in longitudinal and in transverse fission, separates the two old nuclei, so that each daughter cell receives the whole of one of the old nuclei, and the binucleated condition has to be reestablished by the prompt division of this nucleus into two. The Opalinid condition seems more archaic, the Euciliate condition more advanced.

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[24] Biological Bulletin, vol. 23, 1912.

Thus far we have discussed chiefly numerical relations in the nuclear conditions of the Opalinidae. The structural and physiological phenomena also are noteworthy. In many Protozoa we can distinguish two sorts of chromatin, which have been called trophochromatin and idiochromatin, or nutritive and reproductive chromatin. In the Euciliata one nucleus, the larger, has the nutritive chromatin greatly developed, leaving, so far as we can see, no unmodified reproductive chromatin present. In the small nucleus, on the other hand, we do not demonstrate the presence of any specialized trophochromatin. All seems to be idiochromatin. Apparently trophochromatin is derived from idiochromatin, being, in different organisms, more or less hypertrophied and modified in connection with its rôle in metabolism.

In many of the less modified Protozoa, which have centro-nuclei, the nuclear centrosome not only contains achromatic kinetoplasm, but also usually is very rich in chromatin. Outside this nuclear chromocentrosome, in the peripheral layer of the nucleus, we find other chromatin. In some species the peripheral chromatin seems clearly to arise from the chromocentrosome, granules of chromatin arising in this central body and passing out into the peripheral region of the nucleus. We have by no means sufficient evidence to indicate that in all species the chromatin of the peripheral layer is of chromocentrosomal origin. In the mitoses of some of these lower Protozoa we find two distinct sets of chromosomes, one set derived from the chromatin in the chromocentrosome, another set derived from the chromatin of the peripheral region of the nucleus. In some species, at least, these two sets of chromosomes remain distinct throughout the whole mitotic cycle, the centrochromatin going to the caryocentrosomes of the daughter nuclei, the peripheral chromatin remaining peripheral in the daughter nuclei. More study is needed of these phenomena. We do not know enough of the relation of these two groups of chromatin to the chromidia that in so many Protozoa are said to pass out from the nucleus into the cytoplasm. Schaudinn and others have shown that in numerous forms the chromidia are trophic, and that at the time of sexual reproduction these vegetative chromidia are absorbed, the residual chromatin in the nucleus functioning as the orderly carrier of inheritance in the regulated series of sexual phenomena. On the other hand, in other species, the nuclei of the gametes are said to be formed by aggregation of chromidia within the cytoplasm of the parent cell, and the original nucleus with its retained chromatin degenerates and is absorbed into the cytoplasm. The formation of nuclei in the cytoplasm from chromidia has recently been shown by Kofoid (1921) to be very improbable, to say the least.

In comparison with these diverse phenomena in Euciliata and the lower Protozoa, what do we find in the Opalinidae? In the Opalinids we find in each nucleus two sorts of chromatin contained in distinct chromosomes. In the Protoöpalinas and Zelleriellas the conditions are very clear, for the nuclei are large and the number of the chromosomes is small, from 4 to 10. The Opalinid nucleus has no centrosome, but we do find a layered distribution of the nuclear contents, similar to that seen in the Plasmodroma. Just beneath the nuclear membrane lie the large, thin masses of chromatin, which in mitosis form the ribbon-shaped macrochromosomes. A little deeper in the nucleus lie the beadlike granules of chromatin, which in mitosis aggregate into lines to form the granular microchromosomes. Still more central are the achromatic granules and films.

It seems probable that the macrochromatin, peripheral, in Opalinids is homologous with the peripheral chromatin in Plasmodroma; while the microchromatin of Opalinids is homologous with the chromatin of the Plasmodrome caryocentrosome: the Opalinid achromatin being homologous to all the achromatic material of the whole nucleus in Plasmodroma including both that of the caryocentrosome and that of the peripheral zone of the nucleus, leaving out of account of course the plasmatic nucleolus.

The sexual phases of the life cycle of the Opalinids have not been studied with these chromatin distinctions in mind. I had definitely planned to do this before writing this paper, but first the war and now other circumstances beyond control have prevented collecting the material necessary for this study. Previous studies of the sexual and presexual phenomena by Neresheimer (1907) and by myself (1909) and certain observations of Löwenthal (1908) give indication of some of the major relations.

Neresheimer described for *Opalina ranarum* the degeneration of the nuclei in good-sized individuals. It was said that before these nuclei degenerated and were absorbed they gave off into the cytoplasm great quantities of chromidial material, from which gradually new nuclei were formed, at first small, but growing to a larger size. Some time after this process was completed the animals entered upon a period of rapid division, by which they became small and came to contain but few nuclei. These small individuals encysted and, passing out into the water, infected the tadpoles which ingested them. I have found from 1 to 12 nuclei in such infection cysts. The nuclei of the cysts I have found to contain each from one to four, rarely more, rounded masses of dense chromatin lying upon the inner surface of the nuclear membrane. These clumps of chromatin are thrown out into the cytoplasm and are there absorbed. They are the bodies which Neresheimer has regarded as equivalent to polar bodies, but this interpretation seems incompatable with the irregular number of such bodies (one to four or more) which I have found.

Phenomena of nuclear degeneration are present in some of the material used in the studies for the present paper, but they seem to me pathological.

Löwenthal (1908) finds that the chromatin spheres[25] in the cysts stain clear blue with Giemsa's solution, while the larger of the granules in the nuclei stain red. With methyl green in weak acetic acid the spheres stain green. Treated with acetic acid the spheres become highly refractive; then, if ammonia be added they become invisible but are not dissolved. From these observations Löwenthal concludes that the spheres are chromatin, but of a different nature from the chromatin granules. The former he calls cyanochromatin, on the basis of their reaction to Giemsa's stain, and the latter erythrochromatin. The cyanochromatin he regards as corresponding to Schaudinn's somatic chromatin, the erythrochromatin he compares to reproductive chromatin.

Leger and Duboscq (1904, *b*, III) distinguish between the superficial plates and bands of chromatin in the nuclei of *Protoopalina saturnalis*, and other more central chromatin granules which during mitosis become arranged in lines to form chromosomes. Only these granular chromosomes are regarded by Leger and Duboscq as true chromosomes.

The sexual phases of the life cycle have not been studied with the two classes of chromatin in mind, and we can not say with positiveness to what portions of the ordinary vegetative nucleus each corresponds, but from my former studies of *Protoopalina* it seems clear that in *P. intestinalis* and *P. caudata* the chromatin spherules in the cysts are derived from the macrochromosomes, while the larger of the granules in the nuclei of the cysts are the microchromosome granules. These phenomena need restudy with close scrutiny of the relations to the two types of chromatin, but, if the relations stated in the last sentence be correct, then most if not all of the macrochromosome material is thrown out of the nuclei and is absorbed into the cytoplasm before conjugation in Opalinidae, just as the macronucleus of Euciliata is absorbed at a corresponding time. This indicates that the macrochromatin in the Opalinidae is metabolic, is trophochromatin, while the granular chromatin is reproductive, is idiochromatin. Each ordinary nucleus of an Opalinid is then a complete and completely functional nucleus, containing both trophic and reproductive chromatin in full activity. In the Euciliata one of the two nuclei has become specially developed for metabolic activity, being greatly hypertrophied. The other nucleus remains small and for the most part inactive during the vegetative phases of the life cycle. When conjugation occurs only the micronucleus takes part in the sexual process, the hypertrophied metabolic meganucleus being absorbed. Functionally there seems a parallel between the macrochromatin of Opalinidae and the macronucleus of Euciliata, and similarly between the microchromatin and the micronucleus in the two groups. In both groups the hypertrophied metabolic chromatin is absorbed into the cytoplasm when the sexual process occurs, and only the idiochromatin shares in this process.

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[25] These are really disks, not spheres.

Among the lower Protozoa the Plasmodroma, somewhat similar conditions have been described, but there is need of more detailed study before comparisons can confidently be made.

Schaudinn and others have regarded the formation of chromidia in Plasmodroma as the process by which vegetative and reproductive chromatin are separated, and this may well be true. But if so, how shall we regard the processes of chromidia formation in Opalinidae, as described by Neresheimer? In my former studies of Opalinidae I observed degeneration of the nuclei in a few individuals of *Opalina obtrigona* and perhaps in one specimen of *Protoopalina caudata*, but I regarded the phenomena as probably abnormal. All the phenomena are so much easier to follow in the binucleated genera that they should be studied in detail in *Protoopalina*, or preferably in *Zelleriella*, and it is my intention to collect material for this study. In my present material there are three infections of *Zelleriella* which may possibly indicate that in this genus the old nuclei degenerate and new nuclei are formed in the cytoplasm, much as Neresheimer has described for *Opalina ranarum*, but it seems more probable the phenomena are abnormal, perhaps associated with parasites within the Opalinids. Of these three infections in my material, one is in *Zelleriella* [of *Bufo woodhousi*], two others are in *Zelleriella hirsuta* from *Bufo cognatus*. The material is not well enough preserved for satisfactory study of minute detail and, as I hope to get better material, it is not worth while to discuss at any length the phenomena observed. We may note one fact, that in *Z.* [of *Bufo woodhousi*] are found some degenerating nuclei which quite closely resemble the degenerating nuclei of *Opalina obtrigona* which I described in 1909. Raff describes certain "abnormal" individuals of an undetermined species of *Zelleriella* (?), from *Limnodynastes dorsalis*, in which are seen from two to eight nuclei. It seems likely that these are similar to the numerous small nuclei in my infections of *Z. hirsuta* and *Z.* [of *Bufo woodhousi*].

Although some phases of the nuclear phenomena in Opalinidae are still obscure, especially in their interpretation, enough is known to indicate that the structure and behavior of the nuclei in this group are more archaic than in Euciliata. There will be further reference to this point in connection with the discussion of the relationships of the Opalinidae to other groups (section 6).

The nuclei of most species of *Protoopalina* and of all known species of *Zelleriella* are remarkably favorable for study[26] because of their large size and clearly seen chromosomes. In the *Zelleriellas* the flattening of the body is a further aid to study, for in some forms with large nuclei the nuclei occupy the whole thickness of the endosarc, being separated from the observer only by the ectosarc. The animals are already in effect microscopic sections.

5. RELATIONSHIPS AMONG THE OPALINIDAE, AND THE CRITERIA OF SPECIES.

Of the four genera of Opalinidae, *Protoopalina* seems the more archaic. Its cylindrical form is like that of the less modified of the Flagellata. *Zelleriella* may well have been derived from *Protoopalina* by mere flattening of the body. It seems to have arisen after the binucleated condition had been established, for we know no uninucleated species of *Zelleriella*. There are no indications of a polyphyletic origin of the Zelleriellas. In the subfamily *Opalininae* we may again regard in general the cylindrical Cepedeas as the more primitive, and the uniformly flattened Opalinas as derivative. The cylindrical forms are sufficiently distinct to justify placing them in a separate genus, *Cepedea*. The uniformly flattened multinucleated forms, the Opalinas, may perhaps have arisen along more than one line, if we may trust form of body and histological appearance as a guide to relationships in the *Opalininae*. There are at least two series of species intergrading between the cylindrical condition, on the one hand, and the uniformly flattened form on the other. The indications are not decisive, but are sufficient to produce the impression of possible polyphyletic origin of the flattened forms. We do not, however, have sufficient data for subdividing the flattened forms into the several genera which, on the basis of polyphyletic origin, would represent their true genetic relationship. Instead we must continue the old genus *Opalina*, although possibly it is not a true taxonomic entity, but merely a convenient term by which to refer to the uniformly flattened multinucleated species. One intergrading series between the Cepedeas and the Opalinas consists of the species *Cepedea magna* (fig. 117, p. 152), *C. obovoidea* (fig. 118, p. 153), and

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[26] In the autumn of 1906 Prof. Theodor Boveri suggested to me to study *Protoopalina intestinalis* and *P. caudata*, saying that they had the most beautiful nuclei he knew among the Protozoa.

C. madagascariensis (fig. 114, p. 150). Another series includes the species *Cepedea globosa* (fig. 119, p. 154), *Opalina helenae* (fig. 188, p. 216), *O. helenae phyllomedusae* (fig. 189, p. 217), and *O. moreletei* (fig. 190, p. 218). One would not for a moment urge that these two series show the actual derivation of Opalinas from Cepedeas, but they show the existence even to-day of more than one set of intermediate forms and this argues that possibly the flattening by which the Opalinas were derived from the Cepedeas may have taken place more than once. *Opalina virgula* may be a flattened *Cepedea*. It is unique in the character of its endospherules, and its occurrence in India instead of in North America, which is the home of the narrow Opalinas, suggests that it may have arisen independently of the other *Opalinae angustae*.

In this connection we should note again (see p. 175) that twice at least in the family *Opalinidae* cylindrical forms have given rise to flattened forms, for *Zelleriella* doubtless evolved from *Protoopalina*, and *Opalina* doubtless evolved from *Cepedea*.

The genus *Protoopalina* does not grade into the genus *Zelleriella* through any series of species as yet studied, unless it be through *P. ovoidea* (fig. 38, p. 67) and *P. xyster* (fig. 39, p. 68), of which the former is considerably flattened and the latter still more flattened, especially in front, but there are no species of *Zelleriella* that seem to complete the series.

The genus *Protoopalina* does grade into the genus *Cepedea*, so far as the number of the nuclei are concerned. *P. quadrinucleata* has four distinct nuclei, yet from its nuclear character it is clearly a *Protoopalina*. *Prootoopalina axonucleata* may have eight, or even more, nuclei, but although these are rather small, they still seem to be *Protoopalina* nuclei rather than *Cepedea* nuclei. Not only the general appearance of the nuclei, but also the small number of macrochromosomes (4) is *Protoopalina*-like. *Cepedea lanceolata* (fig. 102, p. 137), as described by Bezzenberger, has but four nuclei, occasionally five, but its nuclei show the characteristic *Opalina* condition, with reticulate chromatin net, and large chromatin masses beneath the nuclear membrane. I do not think we have to-day any species which are known to form a true intergrading series of related forms between the genera *Protoopalina* and *Cepedea*, but the probable manner in which the Cepedeas arose from the Protoopalinas does seem to be indicated by the series *P. formosae* (fig. 50, p. 81), *P. quadrinucleata* (fig. 51, p. 82), *P. axonucleata* (fig. 52, p. 83), and *Cepedea lanceolata* (fig. 102, p. 137).

In describing the several species of *Protoopalina* we followed a general order indicated by gradual progress from the uninucleated to the multinucleated condition. I would not claim that the species thus placed in juxtaposition are in every case near relatives. In numerous instances this does not seem true. Groups of related species within the genus *Protoopalina* are described in section 7, in connection with problems of geographical distribution.

Among the Zelleriellas there is less diversity. The genus is a very compact one. Specific distinctions are difficult to make. Diversifying evolution has not gone far in this genus and no attempt will be made to distinguish subgeneric group of species. We will see after considering the geographical distribution of the Opalinidae that there is reason to believe that *Zelleriella* is probably the most recent of the four genera to arise, and it has had less time to evolve into diverse forms than have the other three genera. But apparently *Zelleriella* shows less tendency toward divergent speciation than do the other genera. In the discussion of the taxonomy of the species of *Zelleriella* attention must be given to minute detail. Form and size of the body, length of cilia, denseness of ciliation, position of nuclei, number of chromosomes, relative thickness of endosarc and ectosarc, size and shape of endospherules, and even the general appearance of the cytoplasm, in different cases prove of assistance in distinguishing forms which at first glance seem very similar. Some of these features have to be used with great caution, for they vary with the individual and with the stage in the life cycle observed, if not indeed with the physiological condition. I do not doubt that in some instances in the present studies I have been misled. As already emphasized, it is unsafe to treat taxonomically a good many of the species of the Opalinids without having in mind data from the whole life history. But in the absence of more complete data all that we can do is to scrutinize the phenomena of which we do have knowledge, and exercise the best judgment we can in view of these phenomena. It has seemed surely worth while to undertake this general review of the family upon the basis of the data at hand. Many questions remain unsettled, but the data show us many things of interest, not only about the Opalinidae themselves, but about their hosts and about the geologic history of the regions they inhabit, and we find the data as to the Opalinidae telling us important things about the Ciliata in general.

In the *Opalininae* there are many forms closely similar to one another, especially in the genus *Opalina*. Nuclear characters are not so diverse or so readily observed. We must rely more on other features, some of which, as form and size, vary within the species and with the stage of the life-cycle observed.

The Opalinidae, like very many other groups of organisms, present conditions which make it extremely difficult to say just what may best be considered species, what subspecies, what merely *formae*. It is largely a matter of judgment. It seems very probable that most of the so-called species present diverse races, and the more divergent races of separate species may more or less overlap in their characters. Statistical studies and studies of the whole life-history are probably essential to any adequate taxonomic treatment of a goodly number of the "species" of *Zelleriella*, *Cepedea*, and *Opalina*. I believe the present paper errs, if at all, on the side of too great conservatism in the establishment of new species.

But however uncertain may be some of the conclusions as to classification given in section 3, I hope the actual conditions found are clearly presented. If the data are clear any one may exercise his own judgment and preference as to the manner of expressing them in a taxonomic system. It is too much a matter of the personal judgment or even the individual taste of the student. I can claim no more than this for some of the details of the taxonomy adopted in this case.

It may be well to consider what characters of the Opalinids are the more trustworthy as a basis for taxonomic distinctions:

Form of body.—This is often quite various in a single infection. Yet the character of the different shapes and their grouping about a mean generally give the possibility of distinguishing infections of one species from those of another. Yet these distinctions are very difficult to put into words. A group of drawings is more effective than many paragraphs of verbal description. One must be sure to compare whole infections, and not merely individuals, or he may be seriously misled. A daughter cell just come from longitudinal fission will differ in shape from a daughter cell derived by transverse fission, and each will differ from a full-grown individual. When division is occurring rapidly, as in the presexual phases of the life cycle, the daughter cells may not have time to restore the characteristic shape between divisions, and extremes of divergent form may result. We do not know much of the relative frequency of cross and longitudinal fission, whether these are in constant proportion for the family, or whether species differ in this regard, nor do we know whether there are racial or even individual differences in this respect. General proportions, then, must be studied in whole infections. Thickness of the body, and relative thickness of the anterior and posterior ends of the body, often assist in demarcating species. But there are many more special peculiarities of shape that are safely and conveniently used in specific diagnosis, for example, the presence or absence of posteriorly pointed individuals in the infection, the character of such point when present, whether gradually tapering or abrupt, sharp or rounded at the tip, straight or bent. The character of the bend in the anterior end of the body and the curvature of the body as a whole are also usable distinctions. In general, while no single individual may be specifically distinctive in form, the combination of form characters in the several infections compared is very helpful in indicating specific relations.

Size of body must be used with caution. Here again we must compare infections as a whole, for the daughter cells are smaller than full-grown forms. We must be careful, also, to compare truly comparable infections, from corresponding phases of the life cycle.

Ciliation.—Length of cilia in comparable individuals seems a usable character. We may say the same, in general, of density of ciliation. *Zelleriella hirsuta*, for example, is far more densely ciliated than other species. But one character which we have measured and stated for almost all the species described must be warily used. This is the width of the interval between the lines of cilia at the anterior end of the body and at its posterior end. Probably the interval between the lines of cilia varies somewhat with the nutrition of the individual, well-nourished, fat individuals perhaps having the lines of cilia further apart than they would be in the same individuals if starved and shrunken. Nearly all species of Opalinidae have main lines of cilia running the whole length of the body, with other, shorter lines interpolated between them in the anterior portion of the body. In general, the lines of cilia are about twice as numerous at the anterior end as at the posterior end. In some species, on the other hand, they are about three times as numerous. In a few species they are about four times as numerous in front. But here again caution must be exercised. After transverse fission a posterior daughter may have, for a time, fewer anterior lines of cilia than would a full-grown form. The number of cilia to the micromillimeter in a line of cilia is probably a fairly reliable character for specific distinction. The basal granules stain well with the hematoxylin strains and can often be counted under high magnification without unreasonable difficulty. The basal granules in any line of cilia are, in general, somewhat closer together in the anterior part of the body than they are posteriorly, so corresponding portions of the body must be compared, and one must be careful to compare full-grown individuals and not daughter cells. Unfortunately an accidentally contaminated mounting medium caused most of my slides to fade before I got to the study of this point, so I have not used these probably useful measurements.

Relative proportions of *ectosarc* and *endosarc.* In comparing certain species this character is useful, but we must remember that in some species, such as *Protoopalina saturnalis* (fig. 37, p. 64), which have individuals of two forms, one slender, the other swollen, the relative proportions of the ectosarc and endosarc differ in the two sorts of individuals. In making comparisons we must be careful to choose corresponding individuals. The thickness of the ectosarc is much less at the front end of the body in some species, while in others it is about equally thick over the whole body. Similarly in some species, but not in others, the endosarc is more dense anteriorly and has the endospherules more numerous in this region.

The general appearance of the *cytoplasm* and its alveolation differs in different species, but this probably differs also with the physiological condition. This character influences the observer, perhaps unconsciously, but it should be used with caution.

The size and the shape of the *endospherules* seems a rather constant specific character, though in a few species they are quite diverse in size and somewhat so in form. The endospherules are not well shown with most stains. Iron hematoxylin rightly used brings them out very clearly.

The *position* of the *nuclei* is a helpful diagnostic character in some species of *Protoopalina* and *Zelleriella*. In some species of *Opalininae* the nuclei are more close together in the front end of the body, or at one edge of the front of the body in some Opalinas, while in other species the nuclei are evenly distributed throughout the body.

The *size* of the *nuclei* is a most useful diagnostic character if only one is sure that he is comparing individuals from the same phase of the life cycle. It would not do to compare primary nuclei with secondary nuclei, if it should prove that there are these two sorts of nuclei, as Neresheimer describes. If the numerous small nucleus-like bodies are parasites (*Sphaerella?*), they may lead the unwary observer into no less serious error. There are also indications that nuclear size varies with physiological state, in the *Opalinae* at least. For example, in *O. ranarum* and its several *formae*, nuclei whose macrochromatin is in the form of small scattered granules tend to be larger than nuclei whose macrochromatin shows certain large, disklike aggregations beneath the nuclear membrane. Hegner and Hsiang-Fong Wu (1921) show that in *Opalina* [*larvarum*] there is decrease in size of the nuclei as the young *Opalinae* grow larger. Nuclear size, therefore, can not be used without scrutiny as a specific diagnostic character.

The *shape* of the resting *nuclei* has been emphasized as a readily discerned and apparently reliable specific character. In some of the multinucleated species the dimensions and proportions of the dividing nuclei, say, in the anaphase stage, are distinctive, some species having rather broad ellipsoidal anaphase nuclei, other species very slender spindle-shaped anaphase nuclei, and other species being intermediate in this character.

The *mitotic condition* of the *nuclei* we have also emphasized in describing the species of *Protoopalina* and to a less extent the species of *Zelleriella*. The stage of nuclear mitosis at which the division of the body occurs seems to be specifically constant within rather narrow limits.

Chromosome number, especially the number of the readily observed macrochromosomes, is a most usable diagnostic character in the two binucleated genera. In the multinucleated species the nuclei are small and the chromosomes numerous, so it is difficult to determine the chromosome number.

From this review of the usable specific diagnostic characters it is seen that they are rather numerous and that they will give a large number of combinations of characters. Most species, in consequence, are fairly clearly distinguished. In some instances, however, the taxonomic problem is hopeless, at least to one having but few infections of each species.

In section 7 of this paper, in connection with problems of zoögeography, subgeneric groups of species within the genera *Protoopalina*, *Cepedea*, and *Opalina* are discussed.

6. THE RELATIONSHIPS OF THE OPALINIDAE.

We have discussed the relationships within the family Opalinidae. Now we may consider the relationships of this family to other groups. What is its relation to the Ciliata in general, and especially to the astomatous forms which have often been included among the Opalinidae? What relationships with lower groups of Protozoa are indicated? If we can find light upon these questions, we may find indications of the source and manner of origin of the true Ciliata, which are in some respects the most remarkable of organisms. Let us compare the Opalinidae with both Flagellata and Euciliata and with respect to a series of characters, form of body, structure of body plasma, pellicle, ectosarc, endosarc, ectosarc spherules, endopherules, excretory organs, locomotor organs, kinetic structures, general nuclear condition, trophic chromatin, reproductive chromatin, manner of asexual reproduction, presexual and sexual phenomena, life cycle as a whole, mitotic mechanism, occurrence of mitosis and amitosis, binucleate and multinucleate conditions, chromidia. In what of these features do the Opalinidae resemble the Euciliata, with which they have usually been classed, and in what regards do they differ from them? In what respects do they agree with and differ from the lower Protozoa, the Plasmodroma?

The *body form* is not distinctive. The less modified Opalinids are Euglenalike in shape, so are some Euciliates. The alveolar *cytoplasm* is not distinctive.

The *pellicle* is delicate, thicker than in most Plasmodroma, about as it is in, say, *Paramecium*. It differs from the pellicle of Plasmodroma in general, and agrees with that of Euciliates in being longitudinally spirally furrowed and in having rows of cilia inserted in some, at least, of these furrows. This is a distinctly Ciliate character. In at least some Opalinids which I have observed there are major grooves extending through the length of the body, and secondary striae (probably faint grooves) interpolated between these. The secondary striae are more evident anteriorly, but I have seen them also throughout the body. In numerous species of Opalinids, the primary grooves bear rows of cilia throughout the length of the body, and in addition there are interpolated anteriorly shorter grooves also bearing rows of cilia. These grooves are not the same as the pellicular striae, though they may perhaps arise by more emphatic development of some of the latter. This all recalls vividly the pellicular sculpturing of some of the less modified Euciliates and the manner of insertion of their cilia. The pellicular sculpturing is much more developed in most Euciliates (see Maier, 1903). The Opalinidae, therefore, in the character of the pellicle, diverge from the Plasmodroma and markedly resemble the Euciliata. We should, however, note one group commonly classed among the Flagellata, which has some representatives which are somewhat Opalinid-like in the character of their pellicle and the insertion of their cilia. The genus *Trichonympha* has a pellicle with numerous spiral ridges bearing rows of cilia. To this comparison a return will be made later.

The division of the cytoplasm into *ectosarc* and *endosarc* is general among Plasmodroma and Euciliata and shows nothing distinctive. The inclusions in the ectosarc and endosarc, which in the Opalinidae we have called spherules, and the more diffuse aggregations of paraglycogen (?), can hardly be compared with structures in the Plasmodroma and Euciliata, for our knowledge of the morphology, physiology and origin of these structures is very inadequate throughout the Protozoa.

The *excretory organ* in the Opalinidae is very lowly in its development, consisting of a posterior pore, usually closed but reopening in the same spot, connected with a more or less well-developed vacuole or series of vacuoles along the main axis of the body. The excretory vacuole in some species, especially the flattened ones, may be restricted to the posterior end of the body or may be wholly absent. When well developed it runs as a series of more or less confluent vacuoles along the axis of the body, often almost to its anterior end. It is in close proximity to the nuclei as it passes them, and may be branched in the region of the posterior nucleus. This is a simpler, less definitely formed excretory organ than is found among Euciliates. It is little more than a more or less transient fusion of enlarged alveoles, forming a tube often considerably interrupted and with very irregular outlines. Yet this lowly developed elongated organ suggests comparison with the much more definite excretory tubule, say, in *Hoplitophrya* (Metcalf, 1909) and *Pycnothrix* (Schubotz, 1908). In spite of its vagueness it is of the general type familiar in elongated Euciliata. Its contractions are not definite and regular, but occur occasionally at irregular intervals.

The definite localization of the excretory vacuole and its pore in Opalinids might seem an advance over conditions in the Plasmodroma, but it has been shown that even in Amoeba the excretory vacuole and its pore are in constant relation to a localized portion of the plasma and pellicle (Metcalf, 1910).

The *locomotor organs* are cilia similar to the less modified of the cilia among the Euciliata, and they are inserted in longitudinal, spiral, pellicular grooves, as among the holotrichous Euciliata, the basal granules forming lines beneath these grooves. This is a feature of decided resemblance to the Euciliata. Among the Plasmodroma, the genus *Trichonympha* shows similar longitudinal, spiral lines of "flagella," but these instead of being set along the bottoms of pellicular grooves, are set along the crests of well defined, longitudinal, spiral ridges. The flagella themselves, with their basal granules, seem comparable to the cilia of Opalinidae and holotrichous Euciliates.

The *kinetic structures* in the Opalinidae are developed only in connection with the cilia. They consist of rows of basal granules, one for each cilium, connected longitudinally by minute fibrillae, and also apparently connected laterally from row to row. Among the Euciliata Holotricha we find a somewhat similar condition. Nothing quite like this is known among the Plasmodroma. The nearest approach is in the genus *Trichonympha*, but in this genus there is a distinct kinetic center at the anterior end of the body, much as in *Noctiluca*. The subpellicular kinetic fibrillae are more developed in *Trichonympha* than in the Opalinidae. The Opalinidae have no kinetic center or centers, not even a centrosome within the nucleus. In respect to the character of their kinetic structures they most resemble the Euciliata Holotricha, but lack the centrosome which is observed in the latter during the mitosis of the micronucleus.[27]

There are several striking features in the *nuclear* conditions of the Opalinidae: 1, the presence regularly of two or more nuclei in one body; 2, the exact resemblance of these nuclei to one another; 3, the absence of a nuclear (or any other) centrosome; 4, the permanent persistence of the nuclear membrane, a rather usual Protozoan character among both Plasmodroma and Euciliata; 5, the presence of two distinct sets of chromosomes in each nucleus, each with its own set of chromatic fibrillae; 6, the apparent individuality of chromosomes of each set, at least in the *Protoopalinae;* 7, the absorbing of most or all of the macrochromatin before sexual union, with its consequent interpretation as trophic in contrast with the reproductive microchromatin: 8, the formation of a threefold chromatic spindle from *a*, the macrochromatin fibrillae, *b*, the microchromatin fibrillae, *c*, the achromatic alveolar nucleoplasm; 9, the absence of amitotic division of any of the nuclei, such as is seen in the pinching apart of the macronucleus in Euciliates; 10, the persistent orientation of the two nuclei in the binucleated genera. A pleurinucleate condition, generally confined to a more or less restricted phase of the life cycle, is not rare among the Plasmodroma, and we know a number of species of Euciliata which at times assume a multinucleated condition. Some are usually multinucleated. The Euciliata, in general, however, are definitely binucleate, but so are *Arcella*, *Giardia*, and some other Plasmodroma. The numerical conditions in the nuclei are less distinctive than the structure and functions of the nuclei. The Opalinidae show no approach to nuclear differentiation into a large, trophic nucleus and a small, reproductive nucleus. In this regard they are far removed from the Euciliata. Their possession of distinct sets of trophic chromosomes and reproductive chromosomes, and the absorption of the former before sexual union, parallels physiologically the presence and behavior of trophic nucleus and generative nucleus in the Euciliata. It seems a condition somewhat similar to that which we know among the Plasmodroma, for many species of Plasmodroma have two distinct sets of chromosomes, one derived from the nuclear chromocentrosome and the other from the peripheral layer of the nucleus, but we have not satisfactory data as to the behavior of these two groups of chromosomes in connection with sexual phenomena. The Opalinidae are markedly different from and less specialized than the Euciliata in the structure of their two nuclei and their behavior at about the time of sexual union.

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[27] Recent studies by Rees (1922) have shown that *Paramecium* has a well-developed neuromuscular center near the anterior end of the buccal groove. It is possible that proper fixing and staining would demonstrate a neuromuscular center in Opalinids.

The manner of *asexual reproduction* deserves some emphasis. The Opalinidae reproduce by longitudinal and by transverse fission. The Flagellata have for the most part only longitudinal fission, with the exception of *Platymonas* (unpublished observations by I. F. Lewis[28]), *Prasinocladus* ("*Euglenopsis*," Davis, 1894), *Salpingoeca gracilis* (Kent, 1881–1882), *Salpingoeca polygonatum* (Penard, 1921), *Euglena* (?), and some few other forms among the green flagellates (Algae?), mostly less clearly described. The Euciliata reproduce by transverse fission, except perhaps among the Peritricha, and it is usual to interpret the fission in the Peritricha as in the same morphological plane, for it runs transverse to that surface of the body which bears the cytostome. The Opalinidae show regularly both the Flagellate and Euciliate types of fission.

The *presexual phenomena* in Plasmodromes and Euciliates are probably fundamentally similar. Reduction occurs in each, the full chromosome number being restored by fertilization. The Euciliata, apparently because of their unique nuclear conditions, are peculiar in the presexual and postsexual nuclear divisions and the nuclear metamorphoses. Opalinids, being but temporarily pleurinucleated and having uninucleated gametes, do not show the peculiar presexual and postsexual behavior of the nuclei.

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[28] Noted here with his permission.

Opalinidae differ from Euciliata in general in having male *gametes* which differ markedly in form as well as in size from the female gametes. In *Vorticellae*, to be sure, one sees small motile and large nonmotile gametes, but the differences are chiefly differences of size. Neresheimer (1907) claimed that the sexual phenomena in Opalinidae resemble those of Plasmodroma more than those of Euciliata, but the marked heterogamy sets the Opalinidae apart from both groups. The resemblance is to Rhizopods rather than Euflagellates. It is noteworthy in this connection that in Paramecium the migrant and the stationary sexual nuclei differ in size, the more active being perceptibly the smaller.

The *life cycle*, as a whole, in Opalinidae is similar to that of Euciliata, except, indeed, for the formation of the secondary nuclei if Nereshaimer's account should be accepted. Hartmann (1910) describes for "*Trichonympha hertwigi*" presexual phenomena which closely parallel Nereshaimer's description for *Cepedea* and *Opalina*, but Hartmann's results are so confused in some essential features that the phenomena all need restudy before they can be used for comparison. As we know little, if anything, of sexual phases in the life-histories of Flagellata we can not make comparisons here.

The *mitotic mechanism* in Opalinidae is peculiar in having no centrosomes. Apparently the persistant attachment of each chromosome to both poles of the persistent nuclear membrane by means of threads of chromatin does away with any need of centrosomes as fulcra for the mitotic movements. But other nuclei in both Euciliates and Plasmodromes have persistent nuclear membranes. The structural phenomena and the movements in mitosis in Opalinids are clear, but the manner of their derivation from conditions usual in other groups is not clear. There seem to be no informing comparisons to be drawn with either Euciliates or Plasmodromes.

The occurrence of *amitotic division* in the macronucleus of Euciliates is not comparable to anything in Opalinids, owing doubtless to the unique character of the macronucleus, and its temporary place in the life economy of the organism possessing it.

The *chromidial phenomena* described by Neresheimer (1907) and "confirmed" by Dobell (1907) agree with descriptions of conditions in such Plasmodroma as, for example, Rhizomastigina. They are utterly unlike anything known among the *Euciliata*. On the basis of these conditions, Neresheimer would entirely remove the Opalinidae from the Ciliata. But Kofoid (1921), in an address before the American Society of Zoologists, has shown the inaccuracy of the descriptions of the formation of nuclei from chromidia in several of the most often quoted instances. Phenomena of this sort probably do not occur in any organisms. There is great need of following the presexual phenomena in some binucleated species of Opalinid, in which the relations are less complicated, and studying exactly and in detail the whole series of nuclear phenomena, including especially a study of the trophochromatin and reproductive chromatin. Pending this study we can only say that the undisputed chromidial phenomena in the Ciliates include absorption into the cytoplasm of the trophochromatin (macronucleus of Euciliates, most or all of the macrochromatin of the Opalinids) and that in some Plasmodroma the nuclei in the presexual phases of the life-cycle similarly eliminate trophochromatin. As already emphasized, the presexual phenomena in *Cepedea* and *Opalina*, described by Neresheimer, and the closely similar phenomena described by Hartmann for *Trichonympha*, need careful review.

The less modified Opalinidae, *Protoopalina* and *Zelleriella*, resemble structually the holotrichous Euciliata, except for two features: 1, the unique nuclear condition of the Euciliates, and 2, the absence of cytostome in the Opalinids. The latter is probably secondary and due to living in the alimentary canal of their host, bathed by liquid, predigested food. As to nuclear structure, the Opalinids do not possess two different sorts of nuclei, but they do have two or more nuclei in all phases of the life history except during gametogamy, and this is a condition which might, with modification, serve as a foundation for such nuclear differentiation as has arisen among the Euciliates. We know no other group of organisms which could so readily lend itself to such further differentation. Two things are necessary to produce a Euciliate from a binucleated Opalinid: 1, to have the dividing plane in fission pass between the two daughter nuclei of each pair instead of between the two pairs, thus producing a true and permanent rather than a merely temporary binucleated condition, and, 2, to overdevelop the macrochromatin in one of the nuclei and suppress it in the other.

Among the Flagellates the aberrant genus *Trichonympha* most nearly approaches the Opalinidae in structure. Some of its species resemble *Protoopalina* in shape and in locomotor organs, being in form an elongated spindle, and being abundantly clothed with fine cilia arranged in longitudinal, spiral rows, each cilium with its basal granule, and these granules connected longitudinally in lines by delicate neural fibrillae. The anterior neural centers in *Trichonympha*, near the base of the proboscislike organ, seem wholly wanting in the Opalinidae. The cilia of *Trichonympha* differ from those of *Protoopalina* in being inserted along the crests of well developed ridges instead of at the bottom of delicate furrows in the pellicle.

It seems not improbable that the Opalinidae and *Trichonympha* may have arisen from similar ancestors. At least *Trichonympha* shows us among the Flagellata a highly ciliate form like the Opalinidae and the lowlier of the Euciliata. It seems still more probable that the Euciliata arose from ancestors which, like *Protoopalina* to-day, had become disturbed in their relations of mitosis and fission, and that they passed through a similar pseudobinucleated condition to a condition of true binucleation and finally reached their present structure with nuclei of two sorts, one hypertrophied for metabolism, the other inactive except during the sexual period, when it functions in the phenomena upon which inheritance is dependent. The relationship with the Euciliata here suggested may be expressed by the following classification:

Ciliata
  I. Protociliata.
      Opalinidae.
        Protoopalininae.
          Protoopalina.
          Zelleriella.
        Opalininae.
          Cepedea.
          Opalina.
            Opalinae latae (*Orientales*).
            Opalinae angustae (*Occidentales*).
  II. Euciliata.

7. THE HOSTS AND THE GEOGRAPHICAL DISTRIBUTION OF THE SEVERAL SPECIES AND GROUPS OF OPALINIDAE, WITH A DISCUSSION OF THE DISTRIBUTION OF THE FAMILIES AND SUBFAMILIES OF THE ANURA.

In section 3, in connection with each species of Opalinid described, there are given, so far as known, the name of the host, the locality and date of collection, and the name of the collector. Here will be given a condensed table showing the species of Opalinid, the host, the family or subfamily of the host, the known geographical occurrence of the Opalinid, the known geographical occurrence of the host species, the known geographical occurrence of the genus of the host, and, finally, in a separate list, the known geographical distribution of the families and subfamilies of the Anura. If we had extensive knowledge of fossil Anura, reference to their paleogeographic distribution would be given under each genus. As it is, the only reference is under the several families and subfamilies. This will give us a conspectus of the hosts and geographical distribution of the family Opalinidae, and of its genera and species. There have been reported heretofore 55 infections by Opalinids, including several doubtful records and counting the infection of each species and subspecies of host by each species and subspecies of parasite as one unit. This paper adds 173 new infection records, making 228 in all.

Species of Opalinid.	Host species.	Family or subfamily of host.	Known geographic occurrence of Opalinid in the species of host named.	Known occurrence of host.	Known occurrence of genus of host.
PROTOOPALINA.					
P. acuta (Raff)	*Limnodynastes dorsalis* (Gray)	Leptodactylidae	Australia	Australia	Australia, Tasmania.
P. adelaidensis, new	*Hyla adelaidensis* Gray	*Hylinae*	do	South Australia	America, including West Indies; 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia; Papua; Australia.
P. africana, new	*Rana crassipes*, Buchholz and Peters.	*Raninae*	The Cameroons	Western Africa	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
P. australis, new	*Hyla aurea*, Lesson	*Hylinae*	Australia	Australia, Tasmania	America, including West Indies; 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia; Papua and Australia.
P. axonucleata, new	*Bufo bufo asiaticus* (Steindachner).	Bufonidae	Korea, Southern Manchuria.	Temperate Asia	Cosmopolitan, except absent from Australasia and Madagascar.
P. axonucleata, form *lata*, new.	*Rana nigromaculata* Hallowell.	*Raninae*	China	From Korea and Japan to Siam.	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
P. bufonis, new	*Bufo peltocephalus* Tschudi	Bufonidae	Cuba	West Indies	Cosmopolitan, except absent from Australasia and Madagascar.
P. caudata (Zeller)	*Bombina bombina* (Linnaeus).	Discoglossidae	Europe	Across north central Europe.	Europe north to the Baltic, China, Korea.
P. caudata	*Bombina pachypa* (Boulenger).	do	do	Across south central and southern Europe.	Europe north to the Baltic, China, Korea.
Do	*Bufo viridis* Linnaeus	Bufonidae	Europe (in this host reported only from Naples, Italy)	Central, eastern, and southern Europe, through temperate Asia to China.	Cosmopolitan except absent from Austalasia and Madagascar.
P. caudata discoglossi, new	*Discoglossus pictus* Otth	Discoglossidae	Sardinia, eastern Algeria	Northwestern Africa, Sicily, Sardinia Corsica, Southern and Western Iberian Peninsula.	Western Europe and northern Africa.
P. diplocarya, new	*Eleutherodactylus leptopus* Bell.	*Leptodactylinae*	Patagonia	Patagonia, Chile	Tropical America.
P. dorsalis (Raff)	*Limnodynastes dorsalis* (Gray)	do	Australia	Australia	Australia, Tasmania.
P. filiformis, new	*Rana tigerina*, Daudin	*Raninae*	Formosa, Java, Billiton Island near Sumatra.	Eastern Asia and the Malay Islands, Formosa.	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.

P. formosae, new	*Bufo melanostictus* Schneider	Bufonidae	Formosa	India China, Formosa, Malay Archipelago.	Cosmopolitan, except absent from Australasia and Madagascar.
P. hammondii, new	*Scaphiopus hammondii* Baird	Pelobatidae	Wyoming, Utah, Nevada, Mexico.	Rocky Mountains and desert states south into Mexico.	North America, Mexico.
P. hylarum (Raff)	*Hyla aurea* (Lesson)	*Hylinae*	Australia	Australia, Tasmania	America, including West Indies; 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia; Papua, Australia.
P. intestinalis (Stein)	*Bombina bombina* (Linnaeus).	Discoglossidae	Europe	Across north central Europe.	Europe north to the Baltic, China, Korea.
Do.	*Bombina pachypa* (Boulenger).	do	do	Across south central and southern Europe.	Do.
Do.	*Bufo calamita* Laurenti	Bufonidae	? [Brumpt, 1915]	Western Europe from British Isles to Southern Sweden and Gibraltar, east through Germany.	Cosmopolitan (except Australasia, Madagascar).
Do.	*Bufo mauritanicus* Schlegel	do	Northern Africa? [Brumpt, 1915].	Northwestern Africa, Algiers, Morocco.	Do.
Do.	*Discoglossus pictus* Otth (?)	Discoglossidae	Europe (?) [Brumpt, 1915]	Northwestern Africa, Sicily, Sardinia, Corsica, Southern and Western Iberian Peninsula.	Western Europe.
Do.	*Pelobates cultripes* (Cuvier)	Pelobatidae	France	Southwestern Europe	Europe from France to Poland and from southern Sweden to northern Italy, southeast through Asia Minor and Syria to Northwestern Persia.
Do.	*Rana esculenta* Linnaeus	*Raninae*	Switzerland	Central Europe from southern Sweden, south into Italy.	Northern hemisphere, Africa, East Indies; 1 species in northernmost Australia, 1 in northern South America.
Do.	*Triturus vulgaris* (Linnaeus).	*Salamandrinae*	Europe	Europe (except Iberian Peninsula), Western Asia.	Europe, temperate Asia.
P. intestinalis (Stein) (?)	*Hyla aurea* (Lesson)	*Hylinae*	Australia	Australia, Tasmania	America, including West Indies, 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia; Papua, Australia.
Do.	*Hyla ewingii* (Duméril and Bibron).	do	do	Australia	Do.
Do. (?)	*Uperoleia marmorata* Gray	Leptodactylidae	do	do	Australia.
P. longinucleata, new	*Telmatobius jelskii* (Peters)	do	Ecuador	Ecuador	Western South America.
P. macrocaudata, new	*Bombina orientalis* (Boulenger).	Discoglossidae	Southern Manchuria, Korea.	China, Korea	Europe north to the Baltic, China, Korea.
P. mexicana, new	*Scaphiopus multiplicatus* (Cope).	Pelobatidae	Mexico	Mexico	Temperate North America, Mexico.
P. mitotica (Metcalf)	*Ambystoma tigrinum* (Green)	*Ambystominae*	Nebraska (United States of America).	Across temperate North America to central Mexico.	North America, Mexico, 1 species Siam.
P. montana, new	*Megalophrys montana* Wagler	Pelobatidae	Java	Malay Peninsula, Java	Malay Peninsula, East Indies, Ceylon.

Species of Opalinid.	Host species.	Family or sub-family of host.	Known geographic occurrence of Opalinid in the species of host named.	Known occurrence of host.	Known occurrence of genus of host.
PROTOOPALINA—contd.					
P. mossambicensis, new	*Rana adspersa* Tschudi	*Raninae*	Mozambique	Southeast Africa	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
P. nutti, new	*Rana nutti* Boulenger	do	British East Africa	British and German East Africa.	Do.
P. orientalis, new	*Bombina orientalis* (Boulenger).	Discoglossidae	Korea, Manchuria	China, Korea	Europe north to the Baltic, China, Korea.
P. ovoidea, new	*Gastrophryne texensis* (Girard).	*Gastrophryninae*	Texas	Texas	Southern United States, Central and South America.
P. papuensis, new	*Hyla dolichopsis* (Cope)	*Hylinae*	Papua	Papua, Duke of York Island.	America including West Indies, 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia; Papua, Australia.
P. pelobatidis, new	*Pelobates fuscus* (Laurens)	Pelobatidae	Austria	Central Europe to western Asia.	Central and southwestern Europe, Western Asia.
P. peronii, new	*Limnodynastes peronii* (Duméril and Bibron).	*Leptodoctylinae*	Australia	Australia	Australia, Tasmania.
P. primordialis (Awerinzew).	*Rana nutti* Boulenger	*Raninae*	German East Africa	British and German East Africa.	Northern hemisphere, Africa. East Indies, 1 species in northernmost Australia, 1 in northern South America.
P. quadrinucleata, new	*Rana macrodon* Tschudi	do	Java	East Indies	Do.
P. regularis, new	*Bufo regularis* Reuss	Bufonidae	Gold Coast, West Africa; Tana River, East Africa.	Africa south of the Sahara	Cosmopolitan (except absent from Australasia, Madagascar).
P. rhinodermatos, new	*Rhinoderma darwinii* Guenther.	*Gastrophryninae*	Chile.	Chile.	Chile.
P. saturnalis (Leger and Dubosq).	*Box boops* Linnaeus	Pisces	Mediterranean Sea	Mediterranean Sea	
P. scaphiopodus, new	*Scaphiopus bombifrons* Cope.	Pelobatidae	Colorado; "Black Fork," Wyoming (?).	Rocky Mountains, Northern Texas.	Temperate North America, Mexico.
P. stevensoni, new name	*Bufo regularis* Reuss	Bufonidae	Sudan	Africa south of the Sahara	Cosmopolitan (except absent from Australasia, Madagascar).
P. tenuis (Raff)	*Crinia signifera* Girard	*Leptodactylinae*	Australia	Australia	Australia, Tasmania.
Do	*Uperoleia marmorata* Gray	do	do	do	Australia.
P. xenopodos	*Xenopus calcaratus* Buckholtz and Peters.	*Xenopodinae*	Belgian Congo, Africa	West Africa	Africa south of the Sahara.
P. xyster, new	*Gastrophryne usta* (Cope)	*Gastrophryninae*	Tehauntepec, Mexico	Mexico	Southern United States, Central and Southern America.
ZELLERIELLA.					
Z. antilliensis (Metcalf)	*Bufo marinus* (Linnaeus)	Bufonidae	Jamaica, Bermuda	Northern South America; West Indies (introduced).	Cosmopolitan (except absent from Australasia, Madagascar).

Z. antilliensis (Metcalf) (?).	*Bufo arenarum* Hensel	do	Uruguay	S. Brazil to Buenos Ayres	Do.
Do	*Bufo orbignyi* Duméril and Bibron.	do	do	Uruguay, S. Brazil	Do.
Do	*Leptodactylus prognathus* Boulenger.	*Leptodactylinae*	do		Tropical America from Central America to Buenos Aires, West Indies.
Z. antilliensis (Metcalf) (?) [probably *Z. braielensis*].	*Leptodactylus ocellatus* (Linnaeus).	do	do	Brazil, Argentina	Do.
Z. antillienses (Metcalf) (?).	*Limnomedusa macroglossa* Duméril and Bibron.	do	do	Uruguay	
Z. antillienses (Mecalf) (?) [probably *Z. paludicolae*].	*Paludicola bibronii* (Tschudi).	do	do	South America	Continental America from Mexico to Patagonia.
Z. antilliensis Metcalf (?)	*Pseudis mantidactyla* Cope	do	do	Buenos Ayres	South America.
Z. [*atelopodos*] new (?)	*Atelopus varius* Stannius	*Gastrophryninae*	Costa Rica	Central America, Colombia	Central and South America.
Z. atelopyxena, new	do	do	do	do	Do.
Z. atelopyxena [*stelzneri*], new (?).	*Atelopus stelzneri* Weyenbergh.	do	Paraguay	Uruguay, Paraguay, Argentina.	Do.
Z. binghami, new	*Eleutherodactylus binghami* Stejneger.	*Leptodactylinae*	Peru	Peru	Tropical America.
Z. binucleata (Raff)	*Limnodynastes dorsalis* (Gray).	do	Australia	Australia	Australia, Tasmania.
Do	*Limnodynastes tasmaniensis* (Guenther).	do	do	Australia, Tasmania	Do.
Z. [*boulengeri*], new (?)	*Prostherapis boulengeri* Barbour.	*Raninae*	Columbia (S. Am.)	Northern South America	Ecuador and Columbia, South America.
Z. brasiliensis (Pinto)	*Leptodactylus ocellatus* (Linnaeus).	*Leptodactylinae*	La Plata, Argentina; Rio de Janeiro, Brazil.	Brazil, Argentina	Tropical America from Central Mexico to Buenos Aires, West Indies.
Z. [of *Bufo peltocephalus*].	*Bufo peltocephalus*	Bufonidae	Cuba	Greater Antilles	Cosmopolitan (except absent from Australasia, Madagascar).
Z. [of *Bufo punctatu*], new (?).	*Bufo punctatus* Baird and Girard.	do	La Paz, Calif.; Death Valley, California.	Texas, Arizona, California, Mexican California.	Do.
Z. [of *Bufo spinulosus*], new (?).	*Bufo spinulosus* Wiegmann	do	La Paz, Brazil; Lake Titicaca, Peru.	Western Brazil, Peru, Chili	Do.
Z. [of *Bufo sternosignatus*]. new (?).	*Bufo sternosignatus* Keferstein.	do	Tehuantepec, Mexico	From southern Mexico into northern South America.	Do.
Z. [of *Bufo woodhousi*], new.	*Bufo woodhousi* Girard	do	Utah	Rocky Mountain region from Montana to Texas, east to Missouri.	Do.
Z. bufoxena, new	*Bufo haematiticus* Cope	do	Nicaragua	Nicaragua, south to Peru	Do.
Z. couchii, new	*Scaphiopus couchii* Baird	Pelobatidae	Helotes, Texas	Texas, Mexican California	Temperate North America, Mexico.
Z. cusconis, new	*Eleutherodactylus footei* Stejneger.	*Leptodactylinae*	Peruvian Andes	Peru	Tropical America.
Z. darwinii, new	*Rhinoderma darwinii* Duméril and Bibron.	*Gastrophryninae*	Chile	Chile	Chile.
Z. dendrobatidis, new	*Dendrobates tinctorius* (Schneider).	*Dendrobatinae*	Costa Rica	Costa Rica to Ecuador and eastward to the mouth of the Amazon.	Tropical America.
Z. dendrobatidis	*Dendrobates typographus* Keferstein.	do	Costa Rica, Nicaragua	Central America	Do.
Z. [*engystomopsis*], new	*Engystomops stentor* (Espada).	Bufonidae	Taboga Island (Panama)	do	Central America, Ecuador.
Do	*Engystomops pustulosus* (Cope).	do	Tehuantepec (Mexico)	do	Do.

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ZELLERIELLA - contd.					
Z. hirsuta, new	*Bufo cognatus* Say	Bufonidae	Arizona	Arizona, New Mexico, Texas.	Cosmopolitan (except absent from Australasia, Madagascar).
Z. of *Hyla* [*septentrionalis*], new (?)	*Hyla septentrionalis* Boulenger.	*Hylinae*	Bahama Islands	West Indies, Bahama Islands	America, including West Indies; 3 immigrant species (?) in Europe, temperate Asia, northermost Africa: another (?) in Abyssinia; Papua, Australia.
Z. hylaxena, new	*Hyla pulchella* Duméril and Bibron.	*Hylinae*	Paraguay	Brazil, Uruguay, Paraguay, Argentina.	Do.
Z. hypopachos, new	*Hypopachus variolosus* (Cope)	Gastrophrynidae	Gautemala	Central America	Madagascar, India, Central America.
Z. intermedia, new	*Bufo intermedius* Guenther	Bufonidae	Guanajuato (Mexico)	Mexico	Cosmopolitan (except absent from Australasia, Madagascar).
Z. intermedia cuneata, new	*Bufo valliceps* Wiegmann	do	Brownsville (Texas)	Southwestern Texas, northern Mexico.	Do.
Z. leptodactyli, new	*Leptodactylus albilabris* (Guenther).	*Leptodactylinae*	Porto Rico, Vieques Id. (Porto Rico), Tehuantepec (Mexico).	West Indies, Mexico	Tropical America from Central America to Buenos Aires, West Indies.
Do	*Leptodactylus caliginosus* Girard.	do	Tehuantepec (Mexico)	Tropical America (continental).	Do.
Do	*Leptodactylus gracilis* Duméril and Bibron.	do	do	Mexico, Uruguay	Do.
Do	*Leptodactylus microtis* (Cope)	do	Guatemala	Mexico, Gautemala	Do.
Z. macronucleata (Bezzenberger).	"*Bufo melanostictus* Schneider."	Bufonidae	"Asia"	India, southeastern Asia, Malay Archipelago.	Cosmopolitan (except absent from Australasia, Madagascar).
Z. magna, new	*Leptodactylus typhonius* (Daudin).	*Leptodactylinae*	Venezuela	Venezuela, Eastern South America.	Tropical America from Central America to Buenos Aires, West Indies.
Z. microcarya, new	*Bufo lemur* Cope	Bufonidae	Porto Rico	Porto Rico	Cosmopolitan (except absent from Australasia, Madagascar).
Z. opisthocarya, new	*Bufo coniferus* Cope	do	Nicaragua, Costa Rica	Louisiana through Central America to Colombia.	Do.
Z. opisthocarya [of *Bufo monxiae*], new (?).	*Bufo monxiae* Cope	do	Guanajuato (Mexico), Yucatan.	Mexico	Do.
Z. opisthocarya [of *Bufo typhonius*], new (?).	*Bufo typhonius* (Linnaeus)	do	Panama	Panama, South America	Do.
Z. paludicolae, new	*Paludicola bibronii* (Tschudi)	*Leptodactylinae*	Tolcahuano (Chile)	South America	Tropical continental America from Mexico to Patagonia.
Do	*Paludicola brachyops* (Cope)	do	Venezuela	Panama, Colombia, Venezuela, Northern Brazil.	Do.
Z. patagoniensis, new	*Paludicola bufonina* (Bell)	do	Straits of Magellan (Patagonia).	Patagonia	Do.
Z. ranaxena, new	*Rana draytonii* Baird and Girard.	*Raninae*	California	California and Mexican California.	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.

Z. scaphiopodos, new	*Scaphiopus solitarius* Holbrook.	Pelobatidae	North Carolina (United States of America).	New England to Florida and Texas.	Temperate North America, Mexico.
Z. telmatobii, new	*Telmatobius jelskii* (Peters)	*Leptodactylinae*	Ecudaor, Blanca Island (Peru).	Ecuador, Peru	Ecuador, Peru, Chile.
Z. [*trinitatis*], new	*Phyllobates trinitatis* Garman	*Raninae*	Venezuela	Venezuela, Trinidad	Venezuela, Central America, Cuba, Trinidad.
Z. venezuelae, new	*Hyla venulosa* (Laurenti)	*Hylinae*	do	Central America, South America.	America, including West Indies, 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia; Papua, Australia.
CEPEDEA.					
C. baudinii, new	*Hyla baudinii* Duméril and Bibron.	*Hylinae*	Cordova (Mexico), Guatemala.	Texas, Central America	America, including West Indies: 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa: another (?) in Abyssinia; Papua, Australia.
C. borneonensis, new	*Bufo jerboa* Boulenger	Bufonidae	Borneo	Borneo	Cosmopolitan (except from Australasia, Madagascar).
C. buergeri, new	*Polypedates buergeri* (Schlegel).	*Raninae*	Japan	Japan	Madagascar, Ceylon, India, China, southern Japan, Philippines, East Indies.
C buergeri sinensis, new	*Bufo gargarizans* Cantor	Bufonidae	Hongkong (China)	China	Cosmopolitan (except absent from Australasia, Madagascar).
C. cantabrigensis, new	*Rana cantabrigensis* Baird	*Raninae*	Michigan, Minnesota, Manitoba.	Illinois to Minnesota and north to Great Slave Lake and Saint James Bay.	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
C. cantabrigensis, new	*Rana cantabrigensis latiremis* (Cope).	do	Alaska, Great Slave Lake	From Great Slave Lake to Alaska.	Do.
C. dimidiata	*Bufo bufo* Linnaeus	Bufonidae	Europe	Europe, except Ireland and northwestern Mediterranean Islands, eastward and southward to Amoor River and southern China, northernmost Africa.	Cosmopolitan (except Australasia, Madagascar).
Do	*Bufo viridis* Laurenti	do	do	Middle Europe from Germany south through Mediterranean Islands to northern Africa and east through temperate Asia into China.	Do.
Do	*Rana esculenta* Linnaeus	*Raninae*	do	Central Europe from southern Sweden south into Italy.	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.

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CEPEDEA—continued.					
C. dimidiata	*Rana temporaria* Guenther	*Raninae*	Switzerland	Europe, temperate Asia to Japan.	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
C. dimidiata form *zelleri*	*Rana esculenta* Linnaeus	do	Europe	Central Europe from southern Sweden south into Italy.	Do.
C. dimidiata hawaiiensis, new.	*Rana catesbeiana* Shaw	do	Hawaiian Islands (introduced).	North America east of the Rocky Mountains.	Do.
C. dimidiata orientalis, new.	*Rana nigromaculata* Hallowell.	do	Japan	From Korea and Japan to Siam.	Do.
C. dimidiata paraguensis, new.	*Hyla nasica* Cope	*Hylinae*	Paraguay	Paraguay	America including West Indies; 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia; Papua, Australia.
C. phrynomantidis, new	*Phrynomantis bifasciata* (Smith).	*Gastrophryninae*	British East Africa	Southern Africa	Africa, Amboina.
C. dolichosoma, new	*Bufo haematiticus* Cope	Bufonidae	Nicaragua, Costa Rica	Southern Central America, Colombia.	Cosmopolitan (except absent from Australasia, Madagascar).
C. (?) *flava* (Stokes)	*Scaphiopus solitarius* Holbrook.	Pelobatidae	(?)	Eastern North America from New England to Florida and Texas.	Temperate North America; Mexico.
C. floridensis, new	*Scaphiopus albus* Garman	do	Key West, Florida	Key West, Florida	Do.
C. formosae, new	*Bufo melanostictus* Schneider.	Bufonidae	Hong Kong (China), Formosa.	India, southeastern Asia, Malay Archipelago.	Cosmopolitan (except absent from Australasia, Madagascar).
C. fujiensis, new	*Bufo formosus* Boulenger	do	Mount Fuji (Japan)	Japan	Do.
C. globosa, new	*Phyllomedusa lemur* Boulenger.	*Hylinae*	Costa Rica	Costa Rica	Tropical Central America to Buenos Aires.
C. hispanica, new	*Rana esculenta hispanica* (Michahelles).	*Raninae*	Spain	Spain	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
C. lanceolata Bezzenberger	*Rana nigromaculata* Hallowell.	do	"Asia"	From Korea and Japan to Siam.	Do.
C. longa Bezzenberger	*Rana limnocharis* Wiegmann.	do	"Asia" and Japan, Formosa.		Do.
C. madagascariensis, new	*Megalixalus madagascariensis* Duméril and Bibron.	do	Madagascar (?)	Madagascar	Tropical Africa, Madagascar.
C. madagascariensis [of *Hyperolius*].	*Hyperolius marmoratus* Rapp.	do	West Africa	Angola, West Africa	
C. magna, new	*Bufo latifrons* Boulenger	Bufonidae	The Cameroons (western Africa).	The Cameroons	Cosmopolitan (except absent from Australasia, Madagascar).

C. mexicana, new	*Rana pipiens* Schneider	*Raninae*	Matamoros (Mexico)	North America from Sierra Nevada Mountains east to Florida and New England, and south into Mexico.	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
C. minor, new	*Alytes obstetricans* (Laurenti).	Discoglossidae	Central France	Southwestern Europe	Southwestern Europe.
C. multiformis, new	*Hyla albomarginata* Spix	*Hylinae*	Nicaragua, Bonito (Brazil)	Nicaragua, Brazil	America including West Indies, 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia; Papua, Australia.
C. multiformis [of *Polypedates schlegelii*], new (?).	*Polypedates schlegelii* Guenther.	*Raninae*	Japan	Japan	Madagascar, Ceylon, India, China, Japan, Philippines, East Indies.
C. obovoidea, new	*Bufo lentiginosus* Shaw	Bufonidae	Florida	Eastern United States, east of Texas.	Cosmopolitan (except absent from Australasia, Madagascar).
C. occidentalis, new	*Rana chrysoprasina* (Cope)	*Raninae*	Nicaragua	Nicaragua, Costa Rica	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
C. ophis, new	*Rana tigerina* Daudin	do	Formosa, Billiton Island (near Sumatra).	China, Cochin China, Formosa, East Indies.	Do.
C. pulchra, new	*Kalvula pulchra* Gray	Gastrophryninae	Cochin China	South China, Cochin China, India, Celebes.	India, Southeastern Asia, East Indies.
C. pulchra japonica, new	*Rana rugosa* Schlegel	*Raninae*	Japan	Japan	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
C. pulchra javensis, new	*Bufo melanostictus* Schneider	Bufonidae	Java	India, southeastern Asia, Malay Archipelago.	Cosmopolitan (except absent from Australasia, Madagascar).
C. saharana, new	*Rana esculenta ridibunda* (Pallas).	*Raninae*	Algeria	Europe (except England, Rhine Valley, Denmark, and Italy), Northern Africa, eastward into Turkestan.	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
C. segmentata, new	*Polypedates leucomystax* (Gravenhorst).	do	Cochin China, Java, Sumatra.	Cochin China, Java, Sumatra.	Madagascar, Ceylon, India, China, Japan, Philippines, East Indies.
C. seychellensis, new	*Megalixalus seychellensis* (Tschudi).	do	Seychelles Islands	Seychelles Islands	Tropical Africa, Madagascar.
C. spinifera, new	*Oxyglossus lima* Tschudi	do	Java	Eastern India, southern China, Java.	Southeastern Asia, Java.
C. sp. new?	*Hyla versicolor chrysoscelis* Cope.	*Hylinae*	New Braunfels (Texas)	Arizona, Texas	America including West Indies; 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia; Papua, Australia.
OPALINA.					
O. asiatica, new	*Bufo bufo asiaticus* (Steindachner).	Bufonidae	Shanghai, China	Korea, southern Manchuria, China.	Cosmopolitan (except absent from Australasia, Madagascar).
O. bufoxena, new	do	do	Southern Manchuria	do	Do.
O. camerunensis, new	*Hylambates rufus* (Reichenow).	*Raninae*	The Cameroons, West Africa.	The Cameroons, West Africa.	Tropical Africa.

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OPALINA—continued.					
O. carolinensis, new	*Rana pipiens sphenocephala* (Cope).	*Raninae*	South Carolina, Florida	North Carolina to Florida and Texas, southern Indiana.	Northern hemisphere, Africa, East Indies, 1 species in northernmost Africa, 1 in northern South America.
O. chorophili	*Chorophilus triseriatus* (Wied).	*Hylinae*	North Carolina, Northern Ohio.	North Carolina to Mexican boundary, north to the Great Lakes.	North America north of Mexico.
Do	*Chorophilus occidentalis* (Baird and Girard).	do	Georgia, Florida	Georgia to Florida and Texas.	Do.
Do	*Chorophilus ornatus* (Holbrook).	do	Texas	Texas, Florida	Do.
Do	*Chorophilus feriarum* (Baird)	do	North Carolina, District of Columbia.	Eastern North America	Do.
O. cincta Collin	*Bufo bufo* (Linnaeus)	Bufonidae	France	Europe (except Ireland and northwestern Mediterranean islands (east into temperate Asia (except Asia Minor and Persia), northwestern Africa.	Cosmopolitan (except absent from Australasia, Madagascar).
O. copei, new	*Rana copei* Boulenger	*Raninae*	Costa Rica	Yucatan (Mexico), Costa Rica, Upper Amazon.	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
Do	*Rana pretiosa* Baird and Girard.	do	Montana	Montana north to Puget Sound and south to southern California.	Do.
O. coracoidea Bezzenberger.	*Rana cyanophlyctis* Schneider.	*Raninae*	"Asia"	India	Do.
O. discophrya, new	*Bufo cognatus* Say	Bufonidae	New Mexico	Arkansas to Nebraska, south and west to Arizona.	Cosmopolitan (except absent from Australasia, Madagascar).
O. discophrya	*Bufo copei* Yarrow and Henshaw.	do	Northern and eastern Canada.	East Hudson Bay west to Selkirk settlement.	Do.
O. draytonii, new	*Rana draytonii* Baird and Girard.	*Raninae*	California, Lower California (Mexico).	California, Lower California (Mexico).	Northern Hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
O. gigantea, new	*Bufo compactilis* Wiegmann.	Bufonidae	Texas	Northern Mexico, Texas, Kansas.	Cosmopolitan (except absent from Australasia, Madagascar).
O. guatemalae, new	*Hyla baudinii* Duméril and Bibron.	*Hylinae*	Mexico, Guatemala	Texas, Central America	America including West Indies; 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia; Papua; Australia.
O. helenae, new	*Agalychnis helenae* Cope	do	Nicaragua, Costa Rica	Nicaragua, Costa Rica	Central America.
O. helenae phyllomedusae, new.	*Phyllomedusa dacnicolor* (Cope).	do	Mexico	Mexico	Tropical America to Buenos Aires.

O. hylaxena	*Hyla versicolor* LeConte	do	Michigan	Eastern North America from Canada to Kansas and Texas.	America including West Indies; 3 immigrant species (?) in Europe, temperate Asia and northernmost Africa; another (?) in Abyssinia; Papua, Australia.
O. hylaxena, form *georgiana*, new.	do	do	Georgia	do	Do.
O. hylaxena, form *orbiculata*, new.	do	do	Massachusetts	do	Do..
O. hylaxena parvinucleata, new.	do	do	do	do	Do.
O. japonica, new	*Rana japonica* (Guenther)	*Raninae*	Japan	Japan, China	Northern Hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
O. japonica (?)	*Rana limnocharis* Weigmann	do	Java	Japan, Formosa, Java	Do.
O. kennicotti, new	*Rana areolata* Baird and Girard.	do	Northern Illinois	Indiana and Illinois to Georgia and Texas.	Do.
O. [*larvarum*], new	*Rana clamitans* (?) tadpoles.	do	Nova Scotia	Eastern North America from Florida to Canada, westward into Mississippi Valley and the Great Lakes basin.	Do.
O. lata Bezzenberger	*Rana limnocharis* Wiegmann.	do	"Asia"	Japan, Formosa, Java	Do.
O. moreletei, new	*Agalychnis moreletei* (Duméril).	*Hylinae*	Guatemala	Central America	Central America.
O. natalensis, new	*Phrynobatrachus natalensis* (Smith).	*Raninae*	Sudan (Africa)	Southern and Western Africa	Africa.
O. oblanceolata, new	*Scaphiopus hammondii* Baird.	Pelobatidae	Arizona, Northern Mexico	Mountain States from Nevada south into Mexico.	Temperate North America, Mexico.
O. obtrigona Stein	*Hyla arborea* (Linneaus)	*Hylinae*	Europe	Morocco north to Sweden across Europe and Asia Minor to Japan and southern China.	America including West Indies; 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another in Abyssinia (?); Papua, Australia.
Do	*Hyla arborea japonica* (Schlegel).	do	Manchuria	Japan, Manchuria	Do.
Do	*Hyla arborea savignyi* (Audouin).	do	Jerusalem	Western Asia	Do.
O. obtrigonoidea, new	*Bufo fowleri* Gaman	Bufonidae	North Carolina, Massachusetts.	New England to North Carolina.	Cosmopolitan (except absent from Australasia, Madagascar).
Do	*Bufo punctatus* Baird and Girard.	do	Guanajuato (Mexico)	From New Mexico into Northern Mexico.	Do.
Do	*Gastrophryne carolinensis* (Holbrook).	Gastrophryninae	Georgia, Virginia	South Carolina to Florida, westward to southern Illinois and Texas.	Southern United States, Central and South America.
Do	*Hyla arenicolor* Cope	*Hylinae*	Arizona	Utah and New Mexico to southern California and Mexico.	America including West Indies; 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia; Papua; Australia.
Do	*Hyla femoralis* Daudin	do	Georgia	North Carolina to Florida and Texas.	Do.

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OPALINA—continued.					
O. obtrigonoidea, new	*Hyla pickeringii* Holbrook	*Hylinae*	Illinois	Eastern North America south to South Carolina, west to Illinois and Michigan, north to New Brunswick and Manitoba.	America including West Indies; 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia; Papua; Australia.
Do	*Rana palustris* LeConte	*Raninae*	Ohio, Massachusetts	Eastern North America, west to the great plains and north to Hudson Bay.	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
Do	*Rana* pipiens Schreber	do	District of Columbia, North Carolina, Michigan.	North America east of the Sierra Nevada Mountains.	Do.
Do	*Scaphiopus solitarius* Holbrook.	Pelobatidae	North Carolina	Eastern North America from New England to Florida and Texas.	Temperate North America, Mexico.
O. obtrigonoidea americana, new.	*Bufo americanus* Holbrook	Bufonidae	Ohio, North Carolina	North America east of the Rocky Mountains, south to Mexico, north to Great Bear Lake.	Cosmopolitan (except absent from Australasia, Madagascar).
O. obtrigonoidea americana, form *rugosa*, new.	*Bufo fowleri* Garman	do	North Carolina	New England to North Carolina.	Do.
O. obtrigonoidea austricola, new.	*Rana pipiens austricola* (Cope).	*Raninae*	Costa Rica, Guatemala, Tabasco (Mexico).	Central America	Northern hemisphere Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
O. obtrigonoidea lata, new	*Rana aurora* Baird and Girard.	do	Oregon	Washington, Oregon, northern California, south in the Sierras.	Do.
O. obtrigonoidea lata, new.	*Rana aesopus* (Cope)	do	Florida	Florida	Do.
O. obtrigonoidea maxima, new.	*Bufo boreas* Baird and Girard.	Bufonidae	Western Canada, Alaska	Oregon north to Western Canada and Alaska.	Cosmopolitan (except absent from Australasia, Madagascar).
Do	*Bufo halophilus* Baird and Girard.	do	San Francisco (California)	From San Francisco south to Lower California (Mexico) also California east of the Sierras.	Do.
O. obtrigonoidea orbiculata, new.	*Hyla cinerea* (Schneider)	*Hylinae*	Maryland, Florida, Louisiana, Texas.	Texas, Louisiana, Florida and Maryland.	America including West Indies, 3 immigrant species in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia; Papua, Australia.
O. oregonensis, new	*Hyla regilla* Baird and Girard.	do	Vancouver Island (British Columbia), Oregon.	Pacific Coast of America from Puget Sound to Mexican California, Cascade and Sierra Nevada Mountains.	Do.

O. panamensis, new	*Bufo typhonius* (Linnaeus)	Bufonidae	Panama	Panama, South America	Cosmopolitan (except absent from Australasia, Madagascar).
O. pickeringii, new	*Hyla pickeringii* (Holbrook)	*Hylinae*	North Carolina, District of Columbia, Ohio, Selkirk Settlement.	Eastern North America from Canada to South Carolina, and westward to Illinois, Michigan and the Selkirks.	America including West Indies; 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia; Papua; Australia.
O. raddei, new	*Bufo raddei* Strauch	Bufonidae	China	Eastern temperate Asia	Cosmopolitan (except Australasia, Madagascar).
O. ranarum	*Bombina bombina* (Linnaeus)	Discoglossidae	Switzerland	Across north-central Europe.	Europe, north to the Baltic, China, Korea.
Do	*Bufo viridis* Lauranti	Bufonidae	Europe	Middle Europe from Germany south through Mediterranean islands to northern Africa and east through temperate Asia into China.	Cosmopolitan (except Australasia Madagascar).
Do	*Bufo bufo* Linnaeus	do	do	Europe (except Ireland and Northwestern Mediterranean islands) eastward and southward to Amur River and southern China, Northwest coast of Africa.	Do.
O. ranarum	*Discoglossus pictus* Otth	Discoglossidae	? [Brumpt, 1915]	Northwestern Africa, Sicily, Sardinia, Corsicia, southern and western Iberian Peninsula.	Western Europe and northern Africa.
Do	*Rana dalmatina* Fitzinger	*Raninae*	Bosnia	Southeastern Europe	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
Do	*Rana esculenta* Linnaeus	do	Switzerland	Central Europe from southern Sweden south into Italy.	Do.
Do	*Rana temporaria* Linnaeus	do	Europe	Europe, temperate Asia to Japan.	Do.
Do	*Triturus alpestris* (Laurenti)	*Salamandrinae*	Switzerland	Hills and mountains of Central Europe from Holland to Lombardy, Austria-Hungary, and Greece.	Europe, temperate Asia.
O. ranarum, form *arvalis*, new.	*Rana arvalis* Nilsson	*Raninae*	Lower Austria	Central Europe, Russia, Western Siberia.	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern south America.
O. ranarum, form *cinctoidea*, new.	*Rana temporaria* Linnaeus	do	Germany	Europe, temperate Asia to Japan.	Do.
O. ranarum, form *parvipalmatae*, new.	*Rana temporaria parvipalmata*, Sevane.	do	France		Do.
O. ranarum, form *truncata*, new.	*Rana temporaria*	do	Germany	Europe, temperate Asia to Japan.	Do.
O. ranarum smithi, new	*Bufo smithi* Stejneger	Bufonidae	Japan	Japan	Cosmopolitan (except Australasia, Madagascar).
O. rotunda, new	*Rana erythraea* Schlegel	*Raninae*	Siamese Cambodia	Bombay, Malay Peninsula, East Indies.	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.

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OPALINA—continued.					
O. spiralis, new	*Bufo compactilis* Wiegmann.	Bufonidae	Texas, Arizona	Kansas to Mexico and Peru	Cosmopolitan (except Australia, Madagascar).
O. terrae-mariae, new	*Hyla evittata* Miller	*Hylinae*	Maryland	Maryland, Virginia	America including West Indies; 3 immigrant species in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia: Papua, Australia.
O. triangulata, new	*Bufo lentiginosus* Shaw	Bufonidae	Florida	Southern United States east of Texas.	Cosmopolitan (except Australasia, Madagascar).
O. virgula Dobell	*Polypedates maculatus* (Gray).	*Raninae*	Ceylon	Ceylon, southeastern Asia	Madagascar, Ceylon, India, China, Japan, Philippines, East Indies.
O. virguloidea, new	*Hyla eximia* Baird	*Hylinae*	Mexico	New Mexico, Mexico, Guatemala.	America including West Indies; 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another in Abyssinia (?); Papua, Australia.
Do	*Hyla regilla* Baird and Girard.	do	Los Angeles County (California).	Pacific coast of America from Puget Sound to Lower California (Mexico) and east through the Cascade and Sierra Nevada Mountains.	Do.
Do	*Rana boylii* Baird and Girard	*Raninae*	San Francisco (California)	California, Oregon	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
Do	*Rana sylvatica* LeConte	do	North Carolina, Ohio	Quebec, southwest to the Great Plains.	Do.
O. virguloidea macronucleata, new.	*Acris gryllus* (LeConte)	*Hylinae*	North Carolina	Connecticut to Florida, west to Texas, Kansas and the northwest.	America including West Indies; 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia; Papua, Australia.
O. woodhousi, new	*Bufo woodhousi* Girard	Bufonidae	Arizona, Utah	Rocky Mountain region from Montana to Nebraska, Kansas and Texas.	Cosmopolitan (except Australasia, Madagascar).
O. sp.?	*Bufo haematiticus* Cope	do	Nicaragua	Nicaragua, Costa Rica, Panama, Peru.	Do.
Do	*Hyla septentrionalis* Boulenger.	*Hylinae*	Cuba	Cuba, Bahamas	America including West Indies; 3 immigrant species (?) in Europe, temperate Asia, northernmost Africa; another (?) in Abyssinia; Papua, Australia.

Do.	*Hyla versicolor chrysoscelis* Cope	do.	Texas	Arkansas, Texas	Do.
Do.	*Rana mascareniensis* Duméril and Bibron.	*Raninae*	Goldcoast (Africa)	Africa, Seychelles Islands	Northern hemisphere, Africa, East Indies, 1 species in northernmost Australia, 1 in northern South America.
Do.	*Rana septentrionalis* Baird	do.	Ontario	Adirondack Mountains northeast to Hudson Bay and west to Moose River.	Do.

In the table which occupies the foregoing pages is given the distribution of each genus and species of host known to harbor Opalinidae. There follows a list of the families and subfamilies of the Anura, with a statement of their distribution.

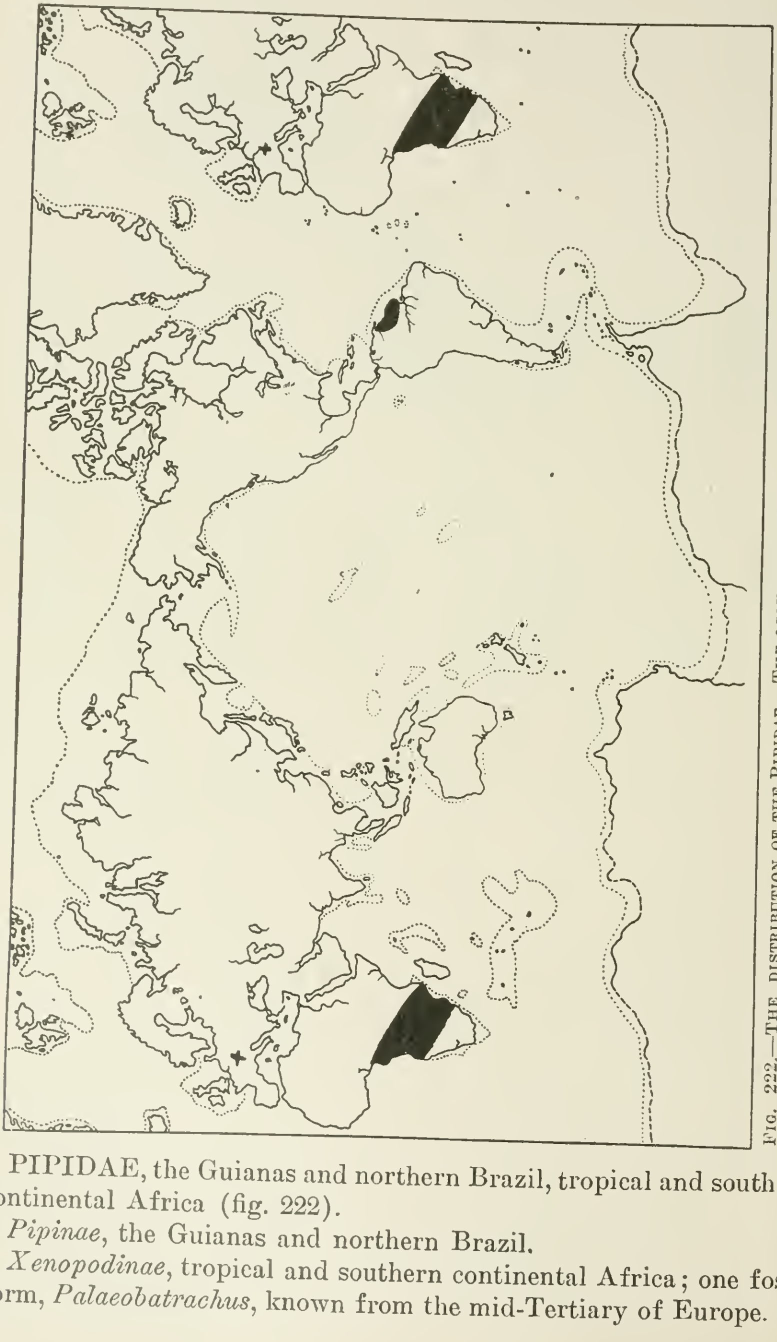

FIG. 222.—THE DISTRIBUTION OF THE PIPIDAE. THE OCCURRENCE OF A FOSSIL PALEOBATRACHUS IN EUROPE IS INDICATED BY A CROSS.

PIPIDAE, the Guianas and northern Brazil, tropical and southern continental Africa (fig. 222).

Pipinae, the Guianas and northern Brazil.

Xenopodinae, tropical and southern continental Africa; one fossil form, *Palaeobatrachus*, known from the mid-Tertiary of Europe.

DISCOGLOSSIDAE, North America (*Ascaphus*, one species in northwestern United States), Europe, southwestern China, and eastern temperate Asia (fig. 223).

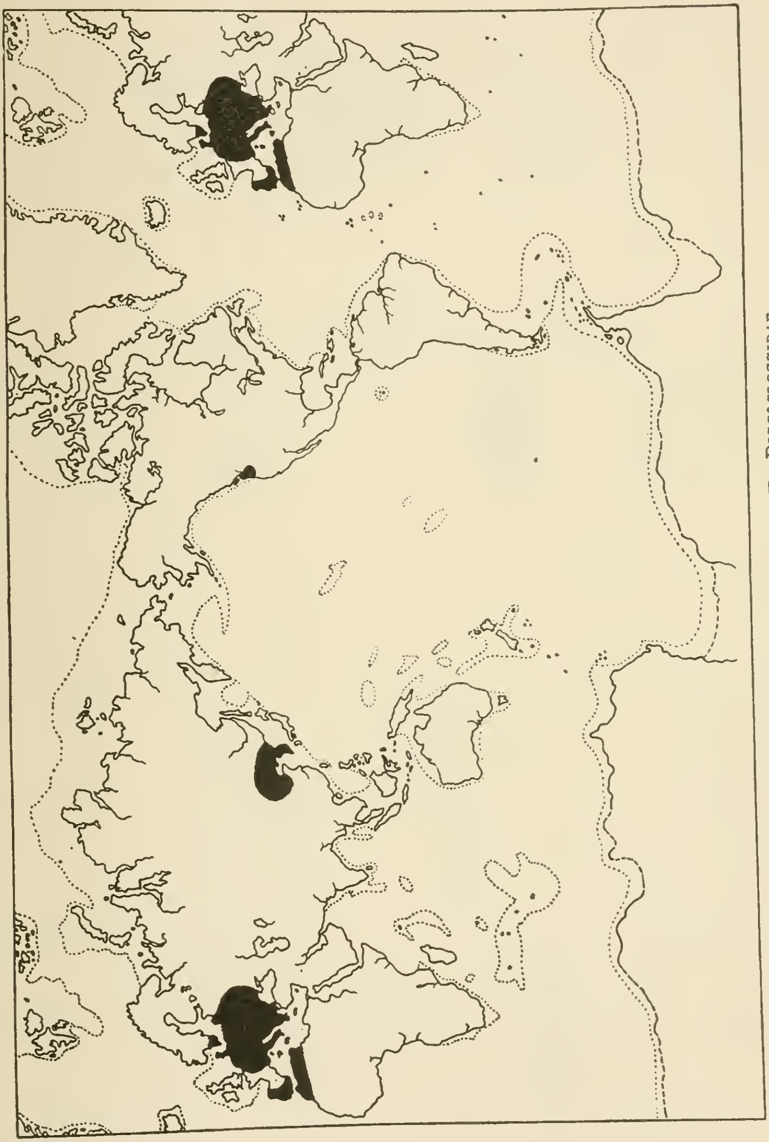

FIG. 223.—THE DISTRIBUTION OF THE DISCOGLOSSIDAE.

PELOBATIDAE, Papua, Malaysia, Ceylon, Asia south of the Himalayas, from the Caucasus and northwestern Persia through southern and central Europe, North America, and Mexico (fig. 224).

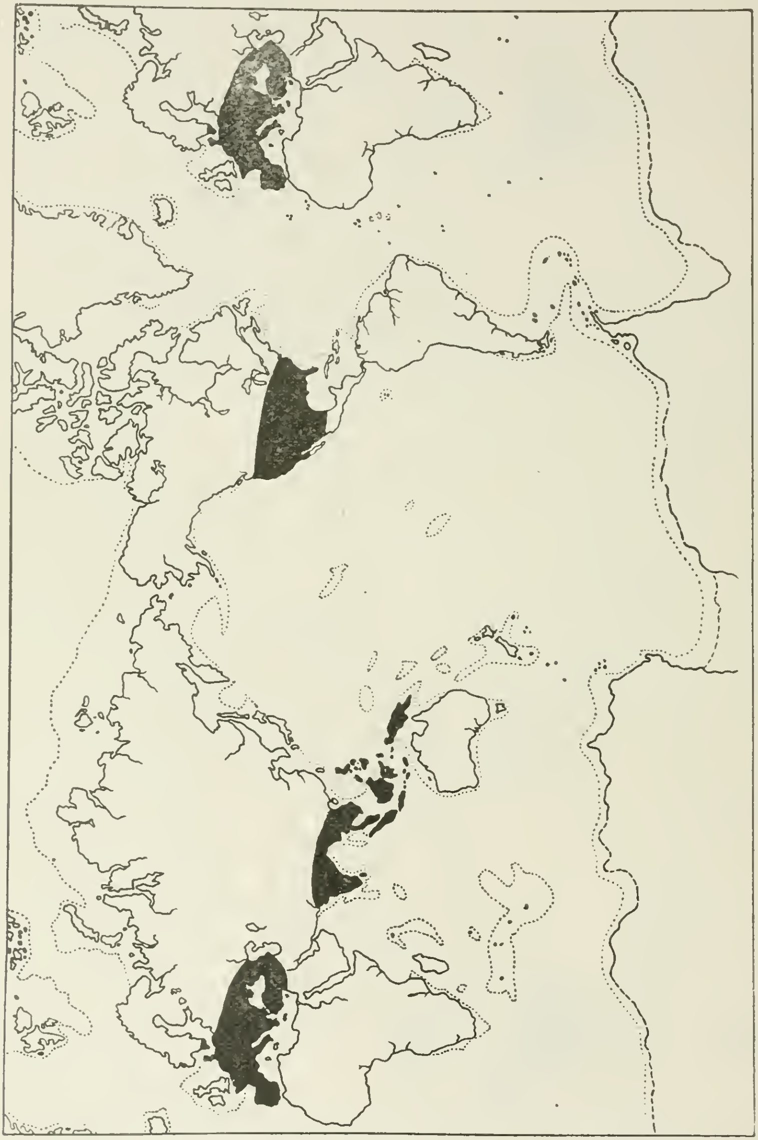

FIG. 224.—THE DISTRIBUTION OF THE PELOBATIDAE.

BUFONIDAE, cosmopolitan within temperature limits, except Madagascar, Papuasia, New Zealand, and the small islands of the Pacific (fig. 225). The genus *Bufo* (fig. 226) has the same distribution as the family except that it is absent from both Australia and Papua. Two Tertiary fossil species, *Bufo serratus* and *B. gessneri*, are reported from Europe.

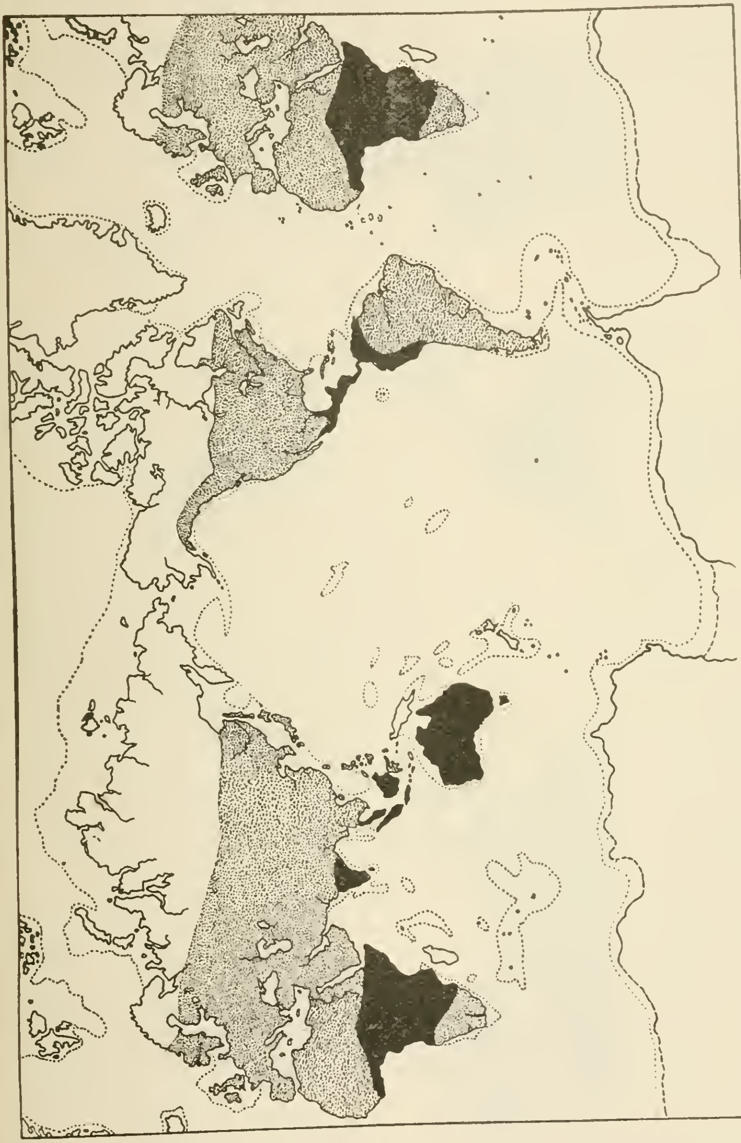

Fig. 225.—The distribution of the Bufonidae. In black is shown the occurrence of genera other than Bufo. The additional territory occupied by the genus Bufo is shown by stippling. Bufo is found in all the areas shown in black except Australia and Tasmania. (See Fig. 226.)

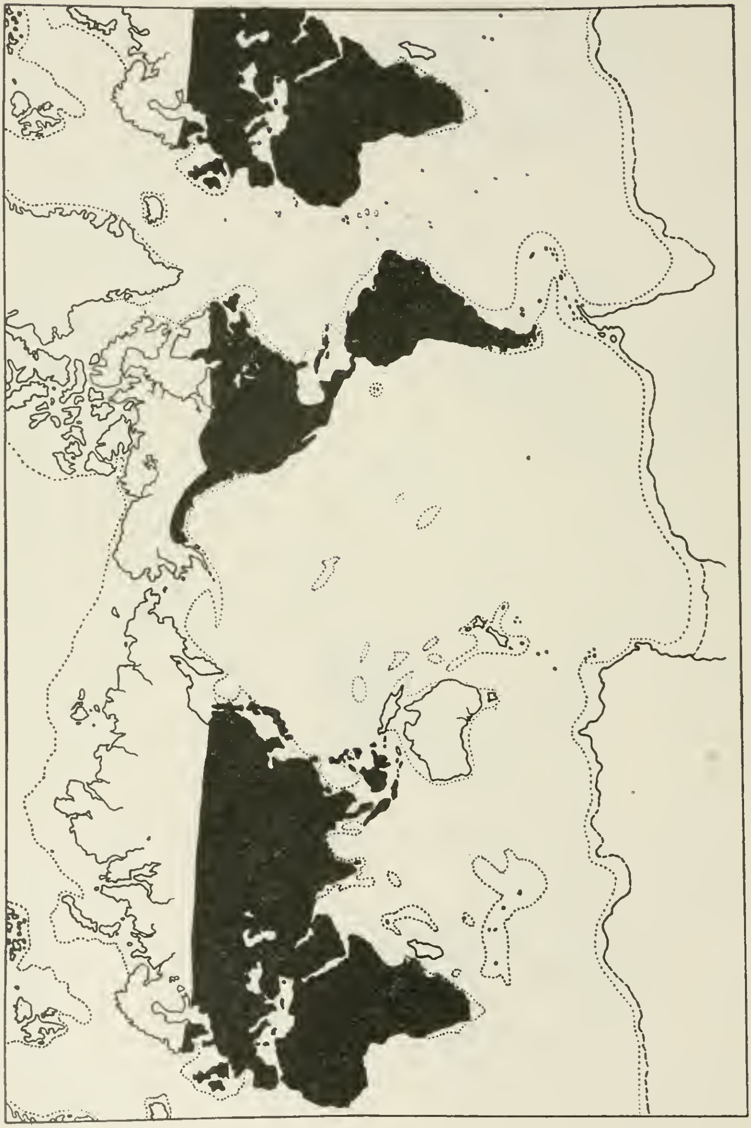

FIG. 226.—THE DISTRIBUTION OF THE GENUS BUFO.

HYLIDAE, predominantly American, mostly from tropical America, but well represented in North America and also in Australia (fig. 227). Several genera, but no species, are common to America and Australia. Three closely related species (or subspecies) are found in temperate Asia, Europe, and northwestern Africa, these being the only Palearctic forms, except a species of *Hyla*, of uncertain affinities which has recently been described from Abyssinia.

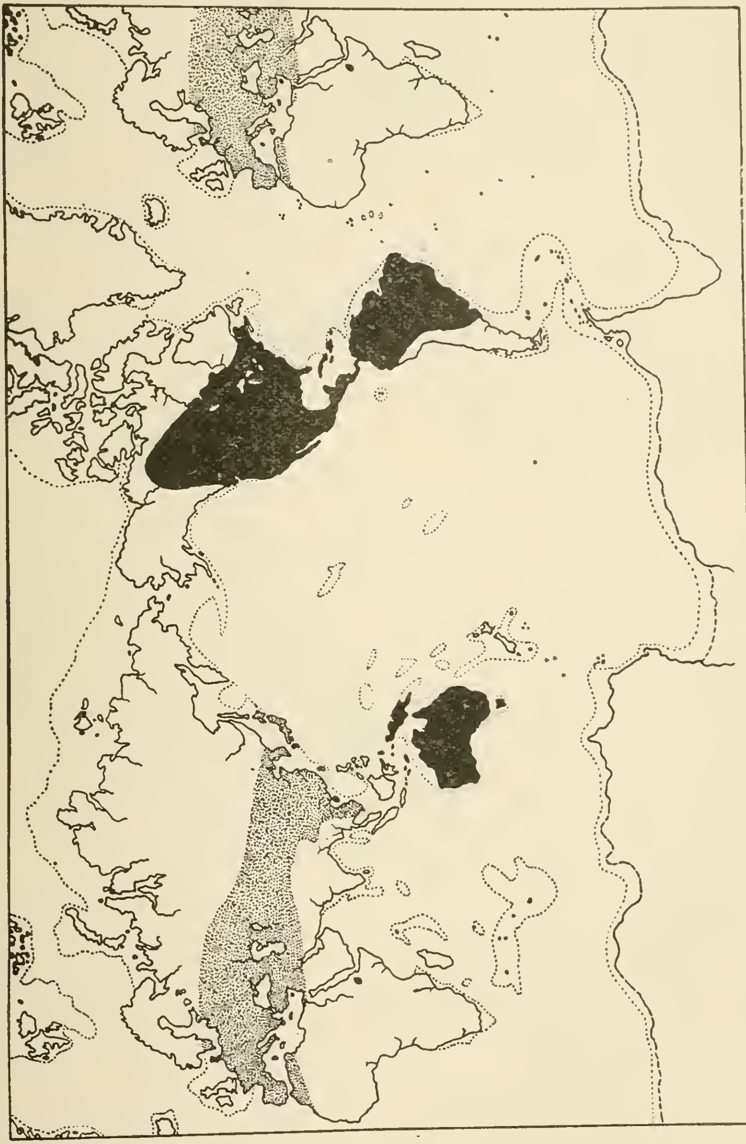

Fig. 227.—The distribution of the Hylidae; the stippled area is occupied only by Hyla arborea and two (three?) closely similar species. The Japanese form is Hyla arborea japonica, and the Abyssinian form, though little known, is probably derived from Hyla arborea.

Amphignathodontinae, one species in Ecuador.

Hylinae, same distribution as the Hylidae.

LEPTODACTYLIDAE, a southern family (probably at least 250 species), known chiefly from South America and Central America, extending north into Texas (two species), and from the West Indies, also well represented in Australia and Tasmania, also one genus *Liopelma* (two species), in New Zealand and one species in Papua (fig. 228). *Heleophryne*, from southern Africa, first described as a Ranid, has recently been assigned to the Leptodactylidae. It may, however, be a Ranid, retaining the "arciferous condition of

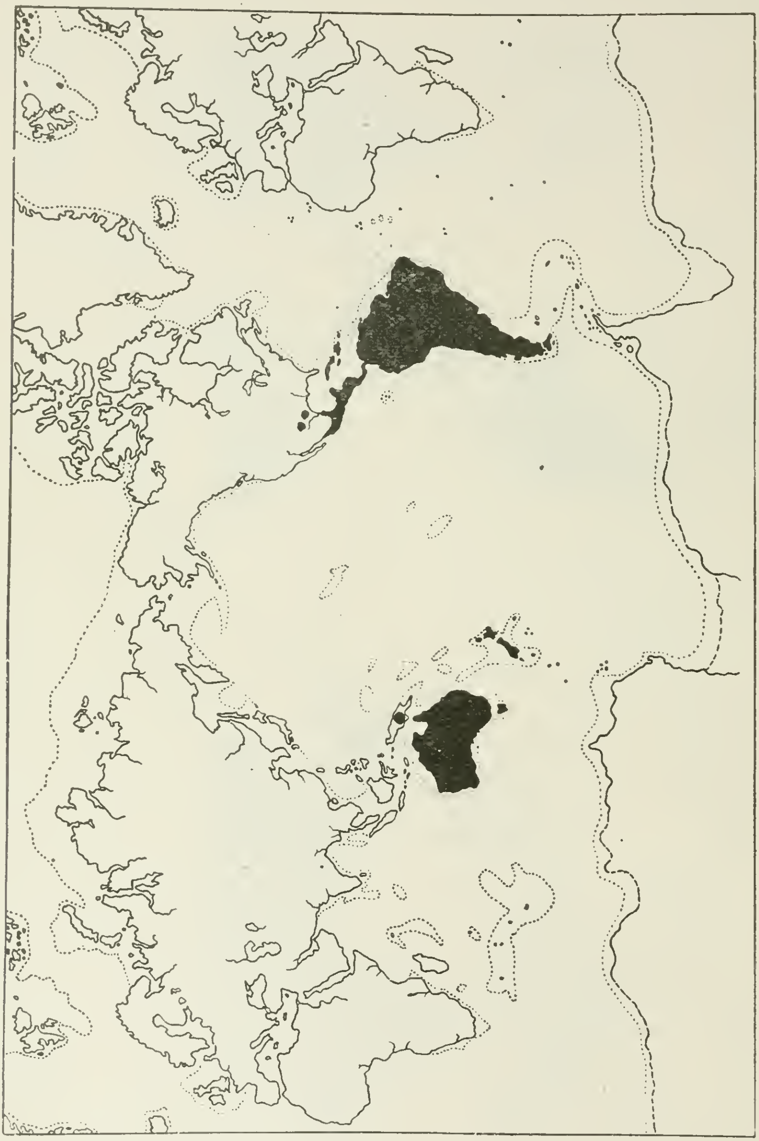

FIG. 228.—THE DISTRIBUTION OF THE LEPTODACTYLIDAE.

the sternum as a result of arrested development." From its distribution I venture the prophesy that it will not finally be accepted as a Leptodactylid.

Hemiphractinae, three genera, all South American.

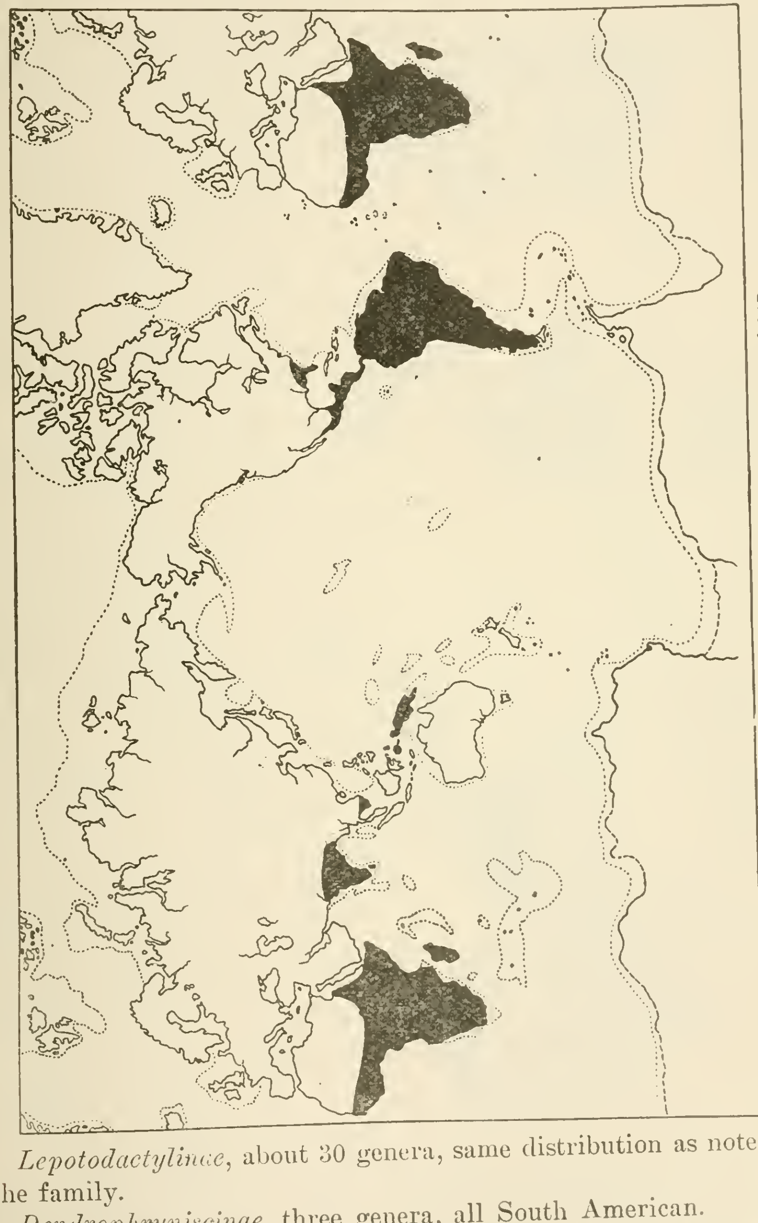

FIG. 229.—THE DISTRIBUTION OF THE GASTROPHRYNIDAE.

Lepotodactylinae, about 30 genera, same distribution as noted for the family.

Dendrophryniscinae, three genera, all South American.

GASTROPHRYNIDAE, tropical America (two genera, three species, extending into southeastern United States), tropical Africa, Madagascar, India, Ceylon, Malaysia, Papuasia, not Australia (fig. 229).

Gastrophryninae, same distribution as noted for the family.

Dyscophinae, eight genera from Madagascar, and three others in India and Malaysia.

Genophryninae, one species in Sudest Island, near Papua.

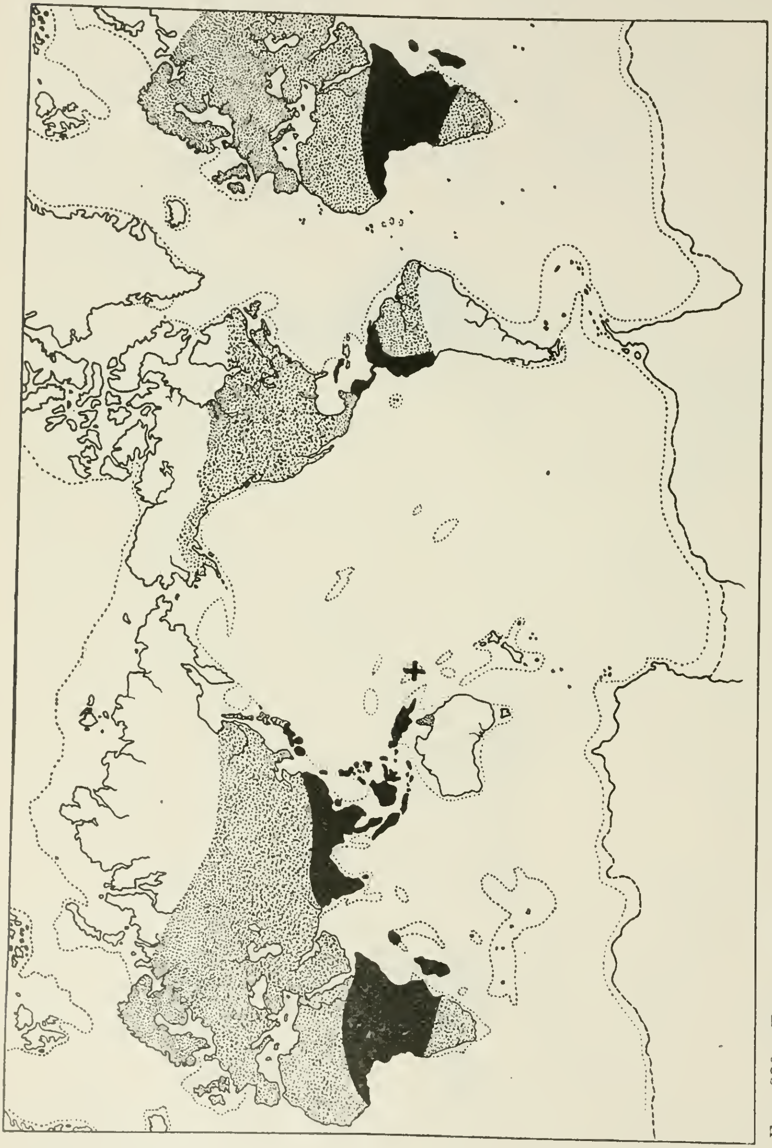

Fig. 230.—The distribution of the Raninae, showing also (black cross) the presence of Ceratobatrachus in the Solomon Islands. The black areas indicate the presence of genera other than Rana. Rana is present in all of these regions except Madagascar and the Seychelles Islands. The gray areas indicate the presence of Rana only. Observe that Polypedates extends to islands in southernmost Japan.

RANIDAE (figs. 230 and 231), cosmopolitan within the limits of temperature, except absent from Australia (one species in the northernmost tip of the continent) and from all of South America except the northern portion, one *Rana* (*palmipes*, the only species of this genus known from South America) extending to Peru and Pernambuco, Brazil (see fig. 230), absent also from most of the oceanic islands.

Ceratobatrachinae (fig. 230, cross), one species in the Solomon Islands.

Raninae, same distribution as the family, except not in the Solomon Islands. One fossil form "*Oxyglossus*" (!), is reported from Wyoming, from "Eocene" (Gadow 1909) or "Comanchian" (Scheubert 1915:) rocks. (Fig. 230.)

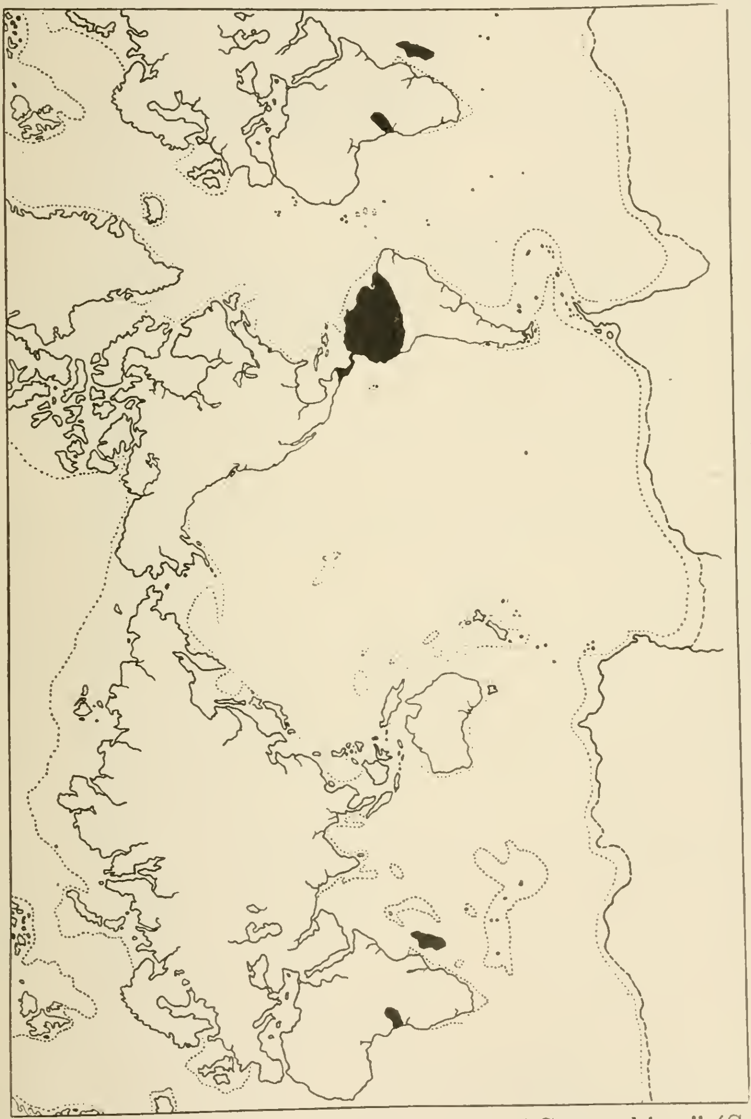

FIG. 231.—THE DISTRIBUTION OF THE DENDROBATINAE.

Dendrobatinae, Madagascar, tropical Africa, Central America and northern South America. (Fig. 231.)

The data in the host-parasite table are significant from several points of view and need to be classified and scrutinized in a number of different ways. One of the things of chief interest will be the geographic distribution. In the discussion of this subject no attempt will be made to treat the data critically from the point of view of geology. The endeavor will rather be to present the known data from the Anura and the Opalinidae and note their implications. Even very scant data, insufficient to have any real weight as they stand, will be stated and their implications noted, with the thought that even very minor items, of slight moment by themselves, may sometime be correlated with other data and then be of interest. The endeavor is, therefore, to have the treatment of this theme inclusive rather than critical.

In such zoögeographical discussion we need a series of paleogeographic charts for reference, and for this purpose I have chosen to follow chiefly Arldt (1907), who draws his conclusions largely from zoögeographic and phytogeographic data rather than from geologic data alone (figs. 232 and 238). Arldt's liberality in accepting biogeographic data at their face value makes his charts the more useful for our present purpose, which is to give freely, rather than critically, the data and indications from our knowledge of the present distribution of Anura and Opalinidae. Modifying Arldt's charts in several points, we will accept them for reference, and thus free our discussion from the necessity of constantly referring to divergent opinions and discrepant data in the fields of geology and biogeography.

Arldt's charts have, however, been modified extensively, especially as to South America and its connections. In the Jurassic, Arldt shows all South America united by a broad trans-Atlantic bridge with Africa (fig. 233, A). We show (fig. 233) central South America occupied by ocean, while the highlands of the Guianas and eastern Brazil are united, the Amazon River not having developed as yet. This northeastern land mass is shown united to northern Africa by a mid-Atlantic bridge. Patagonia, Argentina, and southern Chile, distinct from all other South American lands, form a land mass united to southern Africa by a south Atlantic bridge (von Ihering's Archiplata). These two trans-Atlantic bridges are drawn to follow the course of the shallows (1,000 to 2,000 fathoms) in the Atlantic ocean.

By the time of the early Cretaceous (fig. 234) northwestern South America is shown as connected with westernmost North America and Asia by a strip of land in the eastern Pacific, uniting Ecuador, the Galapagos Islands, the mountain region of middle Central America, the tip of the peninsula of Lower California, the islands off southern California, the coastal ranges of California, Oregon and Washington (including the Santa Inez mountains at Santa Barbara, Presidio Hill and Mount Tamalpais at San Francisco, the Siskiyou Mountains and the Olympic Mountains) and the Alaska islands on to the Aleutian Peninsula and across to Siberia. Puget Sound, San Francisco Bay and San Diego Bay may be remnants of the broad ocean straight running from the Gulf of Mexico to the Arctic Ocean, and separating this eastern Pacific land strip from other North

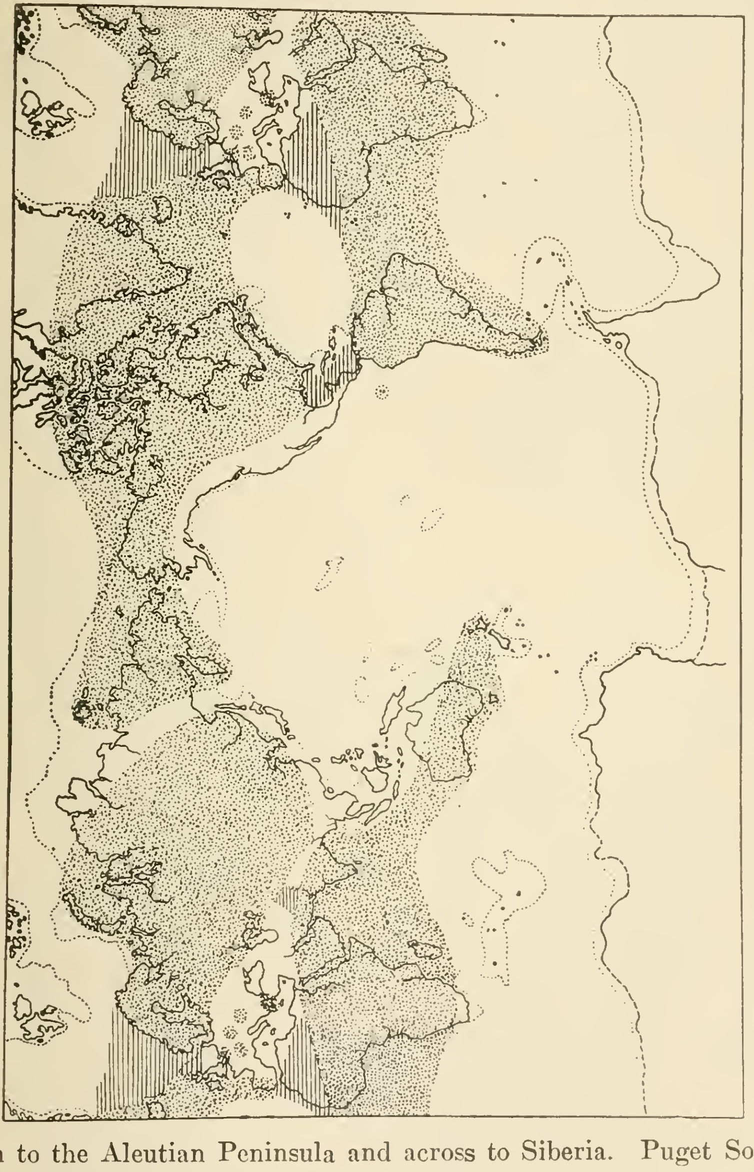

FIG. 232.—TRIASSIC CONTINENTS. (FROM ARLDT.) THE STIPPLED AREAS ARE LATE TRIASSIC. THE LINE SHADING INDICATES ADDITIONAL EARLIER TRIASSIC LAND.

American lands during the Cretaceous period and the earliest Tertiary times. During the later Cretaceous (fig. 235, A) Brazil is shown connected by a mid-Pacific bridge to Australia, while Patagonia at this time or a little later is united to Australasia by the Antarctic route.

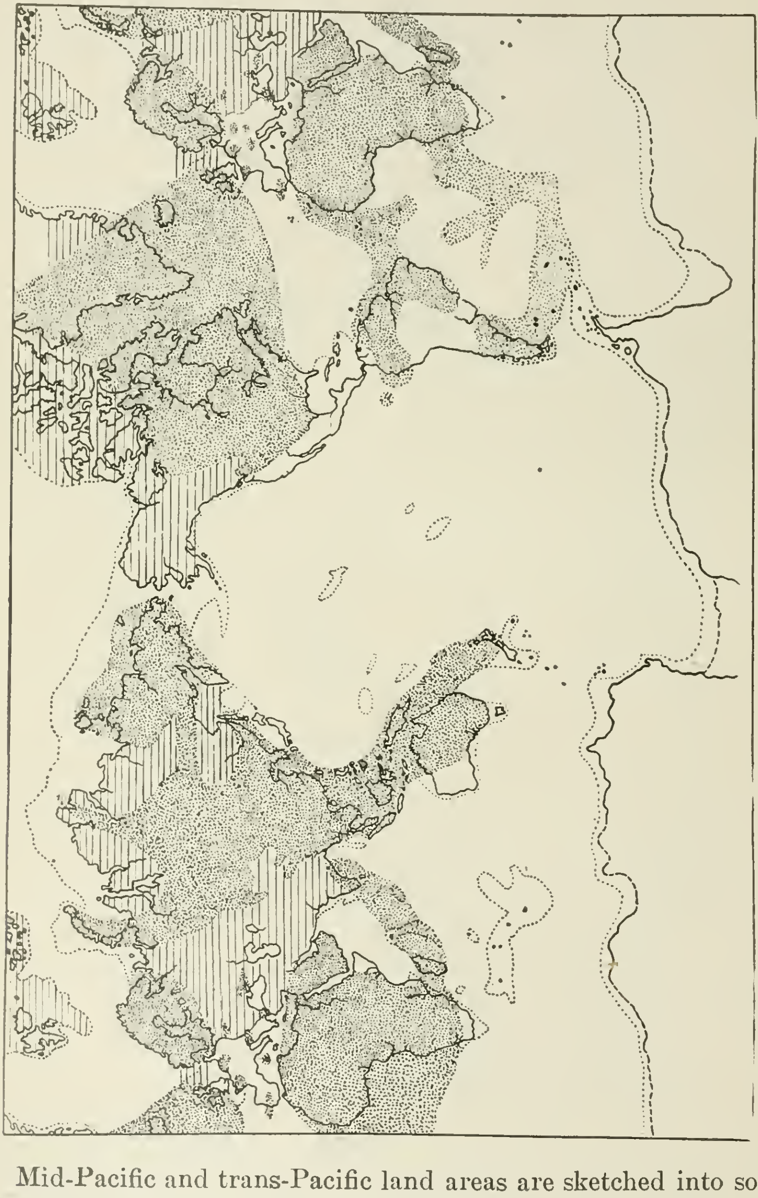

FIG. 233.—JURASSIC CONTINENTS. (FROM ARLDT, MUCH MODIFIED.) THE STIPPLED AREAS ARE LATE JURASSIC. THE LINE SHADING INDICATES EARLY JURASSIC LANDS. ON THE RIGHT THE SOUTH AMERICAN CONTINENT AND ITS CONNECTIONS WITH AFRICA ARE TREATED QUITE DIFFERENTLY FROM THE CONDITIONS IN ARLDT'S CHART (FIG. 233, A).

Mid-Pacific and trans-Pacific land areas are sketched into some of the Cretaceous and early Tertiary maps, following in general the present ocean shallows. Doubtless different Pacific ocean lands were connected in different ways during different parts of these periods, and the patterns of the interruptions of these connections in the different maps are not significant.

Arldt does not show Antarctic connections with Patagonia, New Zealand, or Australia. Our maps show such connection during the later Cretaceous and early Tertiary. An Antarctic route from Patagonia to Australasia seems altogether probable on into Miocene times, at a time when Antarctic fossils show a mild and moist climate. The later Cretaceous may have shown similar connection. Trans-Pacific

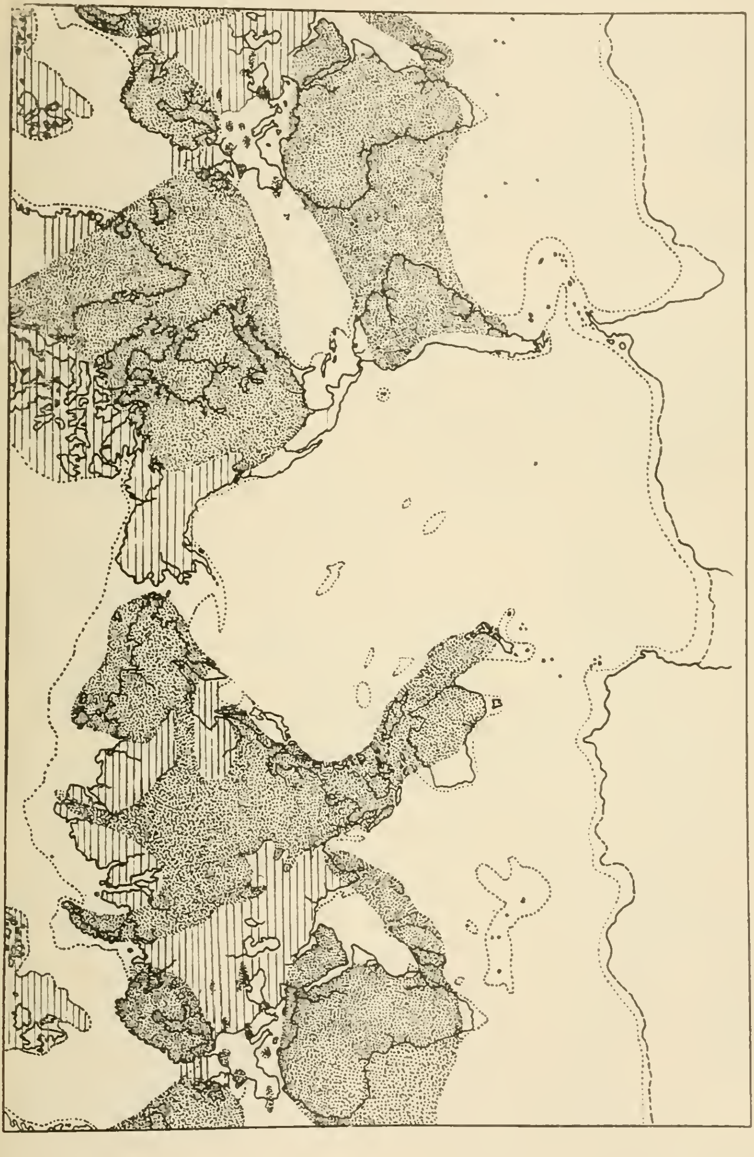

FIG. 233, A.—JURASSIC CONTINENTS. (FROM ARLDT.) THE STIPPLED AREAS ARE LATE JURASSIC. THE LINE SHADING INDICATES EARLY JURASSIC LANDS.

union of the middle portion of South America with Australia is somewhat more problematic, but seems to be indicated by the presence of Hylidae in all tropical America and in Australia and Papua (fig. 227). The absence of Hylidae from southernmost South America today seems to preclude their passage from one hemisphere to the other by the Antarctic route. A trans-Pacific migration seems to be indicated, but its exact route, of course, can not be determined. No Hylidae, or other Anura, are known from any of the mid-Pacific

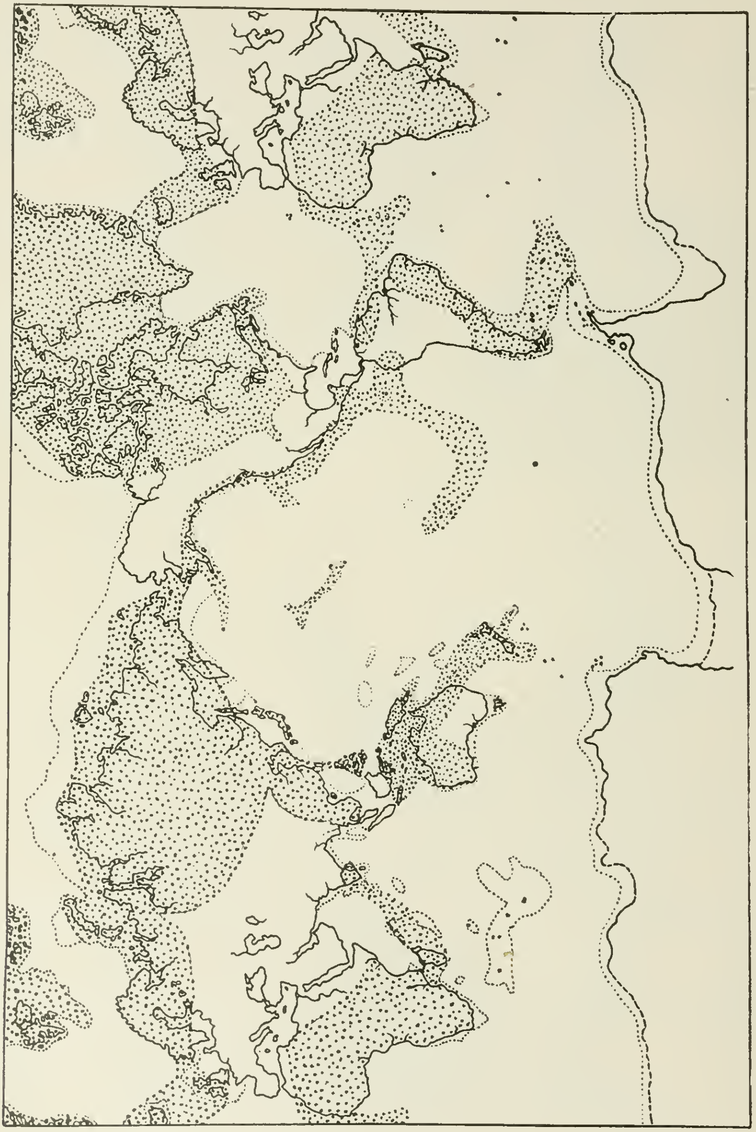

Fig. 234.—Early Cretaceous continents (much modified from Arldt).

islands today, and in New Zealand the only Anura are two species of Liopelma, a Leptodactylid. Arldt's map (fig. 234, A) shows as doubtful a Cretaceous trans-Pacific bridge, south of the Equator, from South America to Australasia, including New Zealand. We have shown this bridge in the late Cretaceous (fig. 235, A), united with central South America, but not with southern South America, and we have not shown it as united to New Zealand. Evidence for these suggestions will appear later in our discussion of the distribution of the Hylidae and the Leptodactylidae (see also page 300).

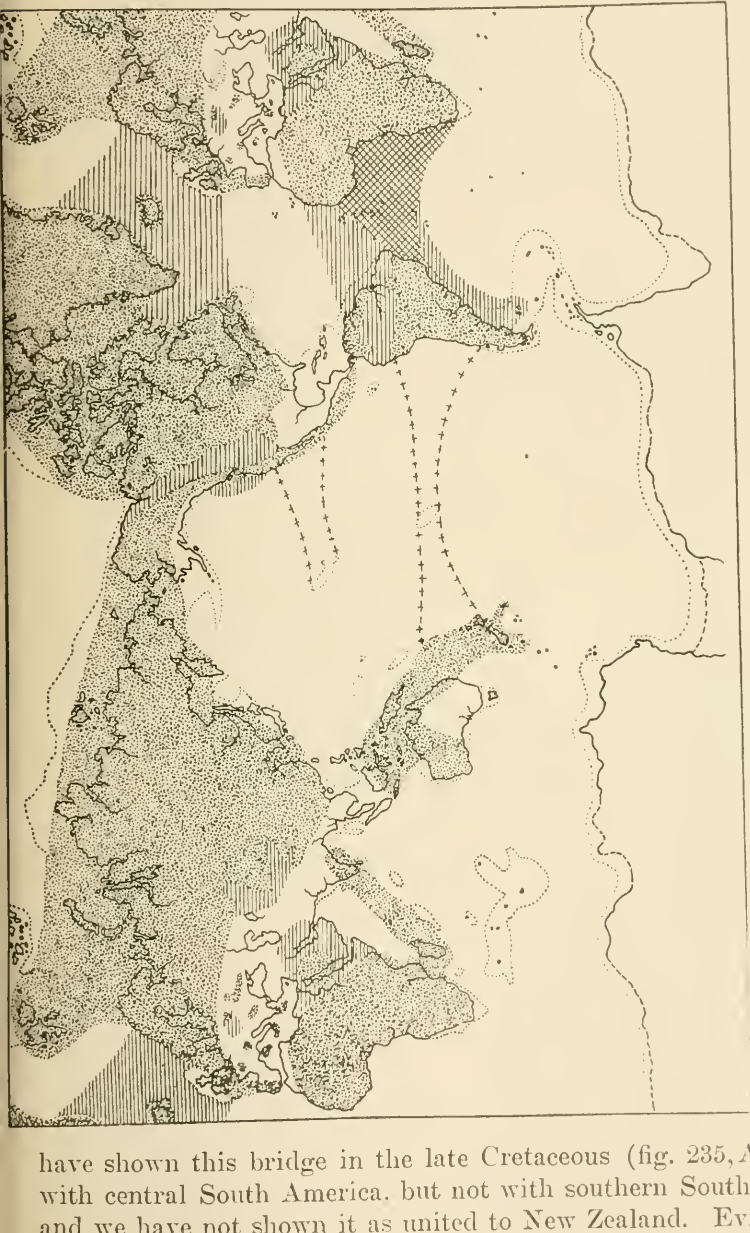

Fig. 234, A.—Cretaceous continents. (From Arldt, modified.) The stippled areas are late Cretaceous lands. The line shading indicates additional Cretaceous lands. The area shaded by crossed lines is von Ihering's (1900) "Archihelenis," accepted by Arldt as late Cretaceous, but rejected in our discussions, following Schuchert (1915?). This chart is misleading unless we note that during the early Cretaceous, Siberia and Alaska were probably separated by Bering Straits and that this gap was not bridged until after the long, narrow ocean strait was formed to the east of the Alaska-California-Ecuador ridge, so that there was no connection between Siberia and North America proper during the Cretaceous.

Arldt's southern Pacific land, "Oceania," is enlarged by Haug (1907–1911) to a great mid-Pacific continent, but our discussion need not take us into the matter of the extent of such Pacific lands. We postulate only a southern Pacific land strip from central and northwestern South America to Australia in the Cretaceous, and an Antarctic union between Patagonia, Australia, and New Zealand in the earlier Tertiary.

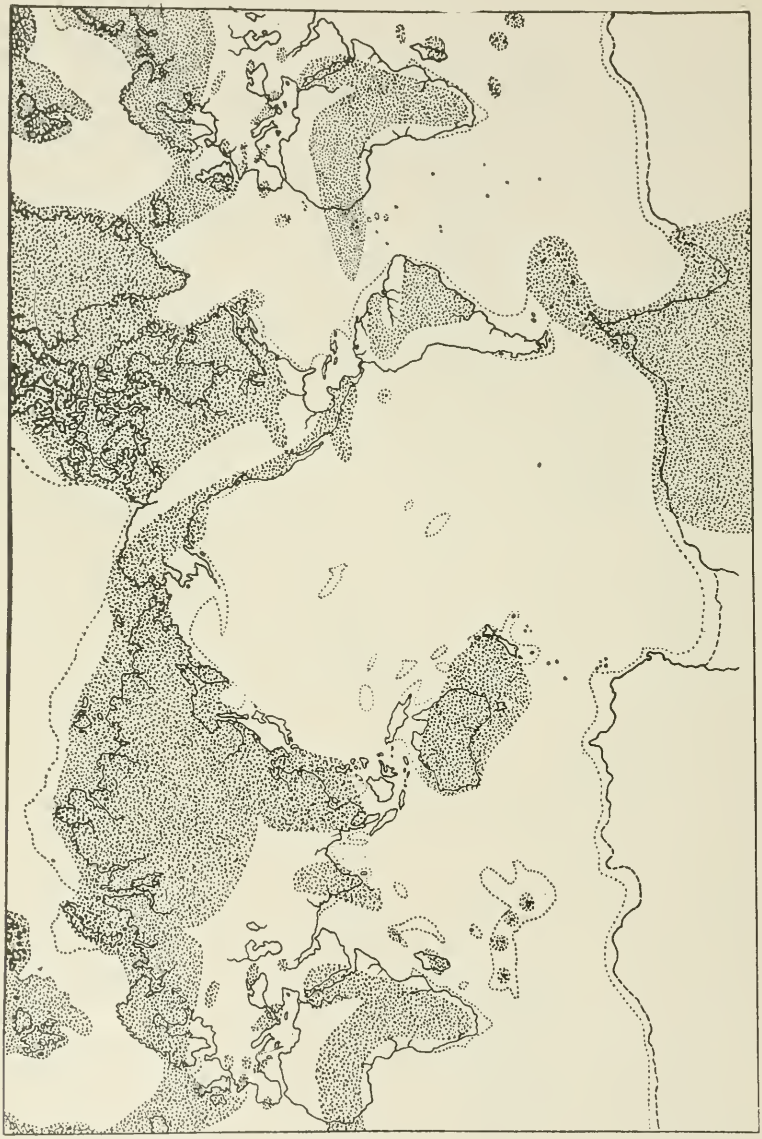

Fig. 235.—Middle Cretaceous continents. (After Schuchert.)

It seems best, in view of varied evidence, to place the final separation of Africa from tropical America in the middle Cretaceous (fig. 235) in agreement with Eigenmann (1909) and Schuchert (1915?), though Arldt shows late Cretaceous and early Tertiary connection between these continents. Again in connection with Arldt's chart of the conditions in the early Tertiary (fig. 236) it must be noted that Patagonia, shown by Arldt as distinct from both tropical America and Antarctica, has been united in our chart with Ant-

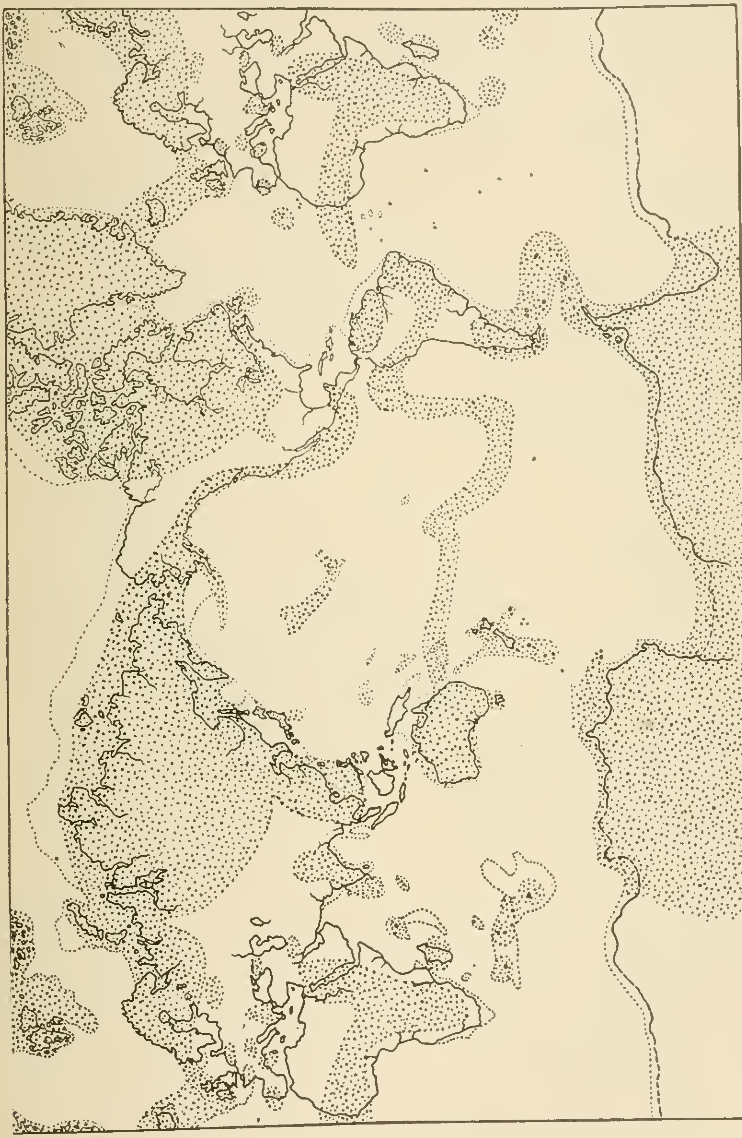

Fig. 235, A.—Late Cretaceous continents. Note the Guianas, eastern Brazil, and Patagonia are distinct from one another.

arctica, and Antarctica has been united with New Zealand and Australia. It is quite possible that there was connection between Australasia and Patagonia via Antarctica in the late Cretaceous.

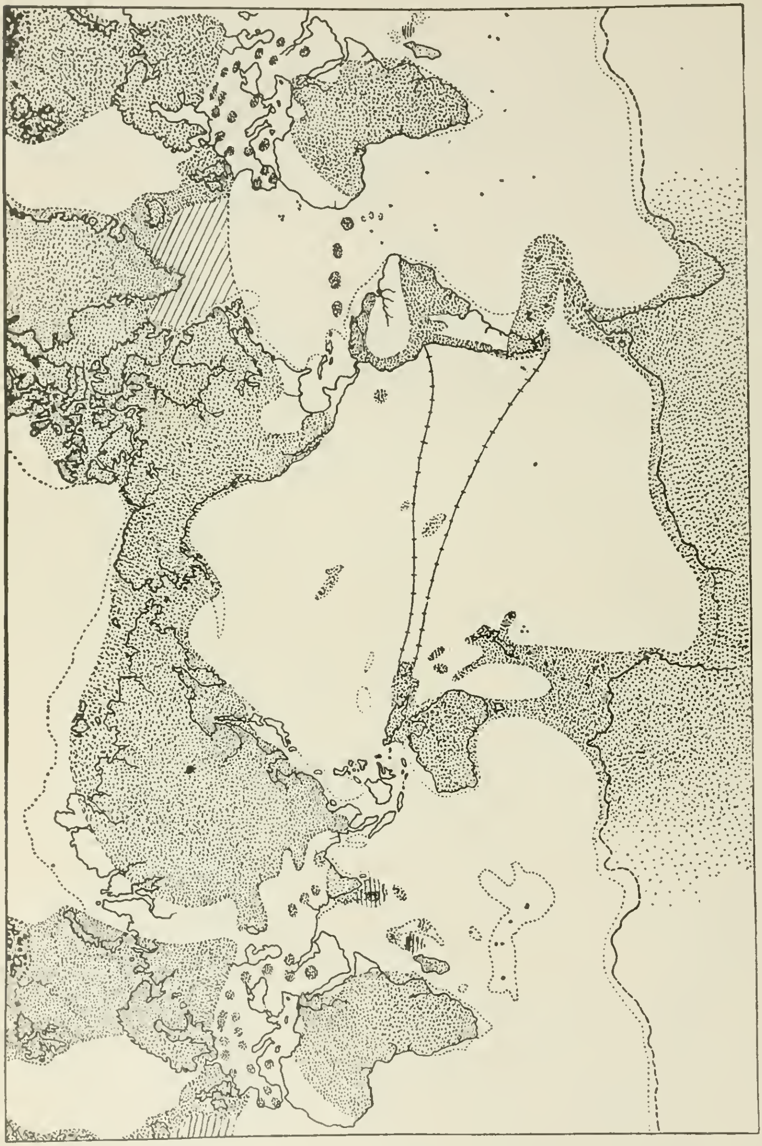

FIG. 236.—EARLY TERTIARY CONTINENTS. (FROM ARLDT, MODIFIED.) THE STIPPLED AREAS ARE OLIGOCENE. THE LINE SHADING INDICATES ADDITIONAL EARLIER LAND AREAS. ANTARCTICA AND ITS CONNECTION WITH AUSTRALIA, NEW ZEALAND, AND PATAGONIA HAVE BEEN ADDED TO ARLDT'S CHART. OUR CHART OMITS A TRANS-ATLANTIC CONNECTION BETWEEN TROPICAL AFRICA AND SOUTHERN SOUTH AMERICA WHICH ARLDT SHOWED FOR OLIGOCENE TIMES.

It should also be noted that there is substantial agreement in the main between Arldt (1907), Haug (1909-1911), and Schuchert (1915?) in such few charts as they show for corresponding geologic periods during Mesozoic and Cenozoic times, though the three authors emphasize to different degrees the several lines of evidence. from sedimentary rocks. from fossils. and from the present distribution of animals and plants.

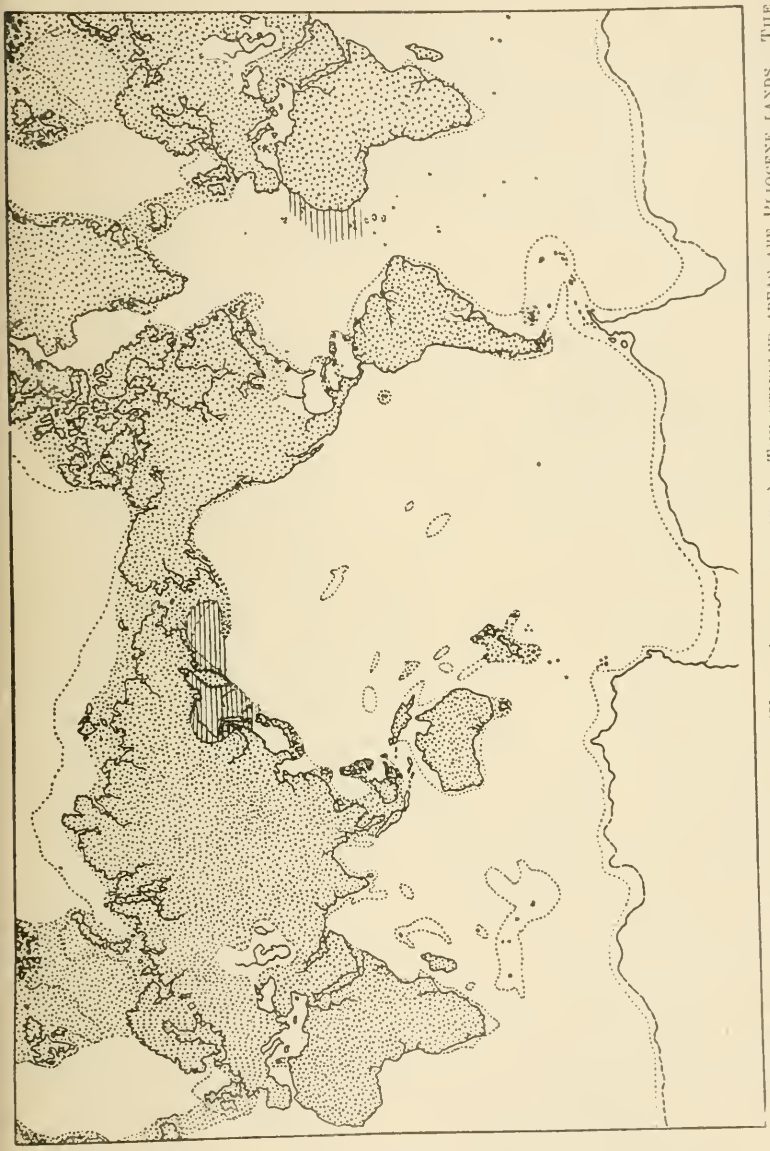

Fig. 237.—Late Tertiary Continents. (From Arldt, modified.) The stippled areas are Pliocene lands. The line shading indicates Miocene lands. Arldt connects the West Indies with Guiana in the Miocene. This chart shows them connected with Central America in the Pliocene (Vaughan, 1919). There is general agreement that Central America south of Mexico was submerged during most, at least, of the Miocene.

There is among paleogeographers rather general agreement that there have been, since the middle Carboniferous period, three chief land masses: 1, An Antarctic continent, Antarctica, connected part of the time with Australia, New Zealand, and Patagonia, and not becoming finally isolated until the middle Tertiary (fig. 237); 2, an equatorial continent, called in this volume Equatoria (fig. 232), uniting New Zealand, Australia, India, Africa, and South America. The Equatorian continent was apparently in existence as early as the Upper Cambrian period. Previous to this period, during the early Cambrian, there were apparently two equatorial continents—Gondwanaland on the east, and to the west South Atlantis, including Africa, South America, and Madagascar. In Jurassic times the

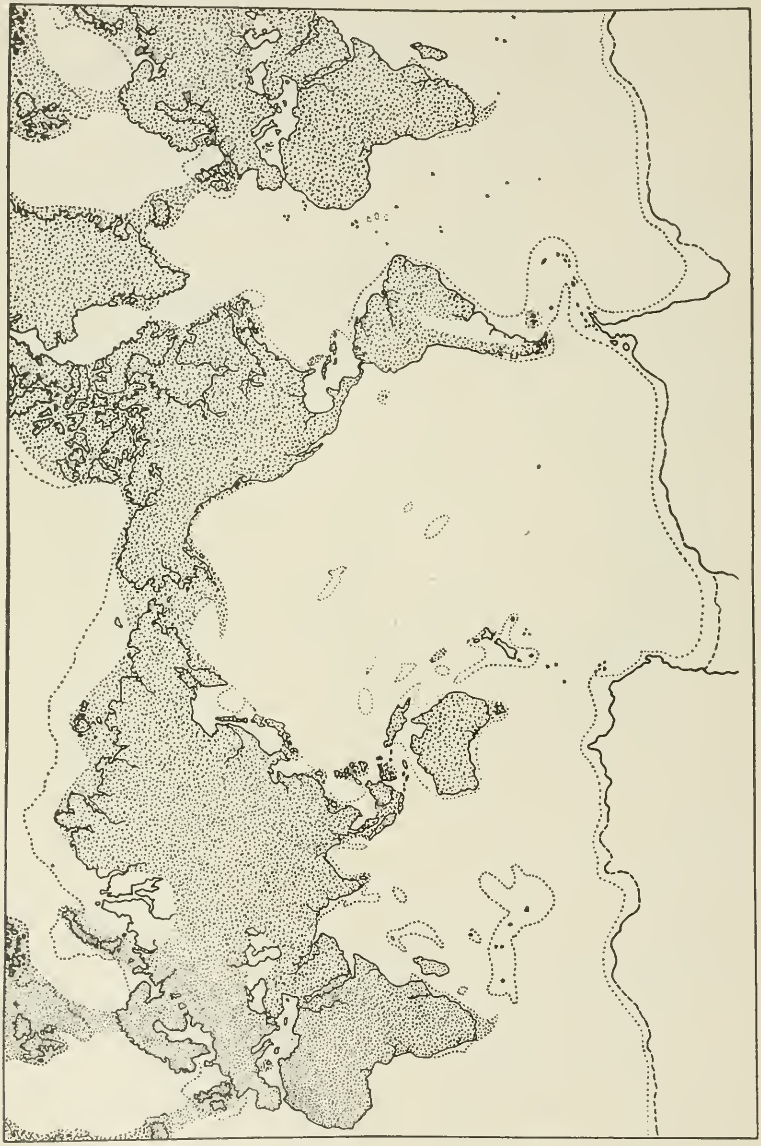

FIG. 238.—PLEISTOCENE CONTINENTS. (AFTER ARLDT.)

Equatorian continent again separated into two parts (fig. 233): Australia parting from the lands to the west and forming instead a temporary connection to the north with Asia-Malaysia; and South Atlantis being reestablished, but with a Madagascar-India extension to the northeast across the Indian Ocean. There was connection between Antarctica and both Australasia and Patagonia after this Jurassic division of Equatoria during the early Tertiary (fig. 236), and perhaps also during one or more earlier periods, though this is not shown in the charts except for the late Cretaceous (fig. 235, A). During the Cretaceous (figs. 234 and 235, A) Africa separated from South America, and at about the same time, or perhaps not until the early Tertiary (fig. 236), the Africa-Madagascar-Seychelles-India bridge became broken up. 3. There have been certain North Temperate and Arctic land masses variously interconnected at different periods, being completely united during most of the Tertiary and during the Quaternary glacial periods (figs. 236, 237, 238). Schuchert (1915?) shows early Permian connections between all continents—Antarctica being united to Australia and South America; Equatoria being broadly connected with Europe; and Europe, Greenland, North America, and Asia all being united into one continuous North Temperate and Arctic land mass. But we are not carrying our discussion of the Anura and the Opalinidae back to the Permian. We have no indication of their existence at that time. This northern land mass, Arctogea, during the middle Tertiary (fig. 237) established connections with the chief remnants of Equatoria (South America, Africa, India, Malaysia [a part of Equatoria in earlier periods but not in Triassic times], but not Australia), and it has retained these connections (fig. 238).

These opinions expressed in the charts used are accepted without criticism as a basis of our present discussion. Even though the paleogeographic conclusions are still under consideration, there is sufficient general agreement on the major features to make it important to align these tentative conclusions with the data in the present paper and to see what suggestions and implications, or even conclusions. may result; and, for the sake of illustrating the host-parasite method of studying zoogeographical and related questions, we will accept and use even the details in the paleogeographic charts chosen. Our discussion is therefore largely of the nature of a preliminary review of the data, subject to later critical emendation.

Of course, in any "geologic period," covering as it did millions of years, there were geographic changes. No series of charts, in the present state of our knowledge, can be expected to show these changes in detail, and we are not even using the most detailed charts available, but are using rather a series of maps which are broad generalizations, omitting many salient phenomena. It is evident that in referring our distribution data in the present paper to the charts selected we are really far from giving the subject adequate treatment. In our discussion we are interested chiefly in intercontinental connections and in climates and the influence of these two factors upon the spread of the forms we are studying, and it is upon these points that critical attention should chiefly be focused. It seems unwise for a zoologist who has little familiarity with paleogeography to attempt critical review of these geographic data. This is purposely left to more competent students, with the expectation that our tentative conclusions may be modified in numerous instances.

In the discussions in this section we will treat the data first chiefly from the point of view of the Opalinidae, then from the standpoint of their Anuran hosts, and later will refer to a number of matters whose discussion must use both viewpoints simultaneously. But this classification can not be followed with any rigidity, for the data from the two groups are so interwoven and their implications are so interdependent that neither aspect of the matters involved can be followed exclusively in any part of the discussion. The tabulation of the detailed infection and distribution data from the point of view of the hosts is placed in the next section, 8, and following this will be given some summary statements.

The Opalinidae are found in the recta or intestines of Anura, Urodela, and Pisces. They are known from one fish only, *Box boops*, from the Mediterranean Sea. Among Urodela they have been reported from three species only, *Triturus vulgaris* [*Triton taeniatus*], and *T. alpestris* from Europe, and *Ambystoma tigrinum* from central North America. All other Opalinids known are reported only from Anura. Only *Protoopalina* is known from hosts other than the Anura, except for Galli-Valerio's (1907) report of an *Opalina* from *Triturus alpestris* (quite possibly a temporary infection). The species in the fish, *Box boops*, is *Protoopalina saturnalis:* the species from *Triturus vulgaris* is *P.* "*intestinalis*" (it is desirable to reobserve this form); the species found in *T. alpestris* is *Opalina ranarum;* the species from *Ambystoma* is *Protoopalina mitotica.* The Protoöpalinas we have regarded as the most primitive of the Opalinidae. It is of interest that it is this most archaic genus which has representatives parasitic in the widest range of hosts. Among the Anura, *Protoopalina* is known from the Pipidae, the Discoglossidae, the Pelobatidae, the Hylidae, the Bufonidae, the Leptodactylidae, the Gastrophrynidae, and the Ranidae; that is, from all the families from which any Opalinids have been reported. Opalinids have not as yet been reported from the following subfamilies of Anura: *Pi-*

pinae, *Hemiphractinae*, *Amphignathodontinae*, *Dendrophryniscinae*. *Dyscophinae*, *Genophryninae*, *Ceratobrachinae*.

The genus *Protoopalina* is reported from—

Pisces (1 species of host, 1 species of *Protoopalina*).
  Box boops (*P. saturnalis*); Mediterranean Sea.
Urodela (2 species of hosts, 2 species of *Protoopalina*).
  Ambystoma tigrinum (*P. mitotica*); Nebraska, United States of America.
  Triturus vulgaris (*P.* "*intestinalis*"); Europe.
Anura.
  Pipidae (1 species of host, 1 species of *Protoopalina*).
    Xenopus calcaratus (*P. xenopodos*); tropical Africa.
  Discoglossidae (5 species of hosts, 6 species and subspecies of *Protoopalina*).
    Bombina bombina (*P. caudata*); Europe.
    Bombina bombina (*P. intestinalis*); Europe.
    Bombina orientalis (*P. macrocaudata*); Korea.
    Bombina orientalis (*P. orientalis*); southern Manchuria, Korea.
    Bombina pachypa (*P. caudata*); Europe.
    Bombina pachypa (*P. intestinalis*); Europe.
    Discoglossus pictus (*P. caudata discoglossi*); southern and western Europe.
    Discoglossus pictus (*P. intestinalis*, doubtful identification); southern and western Europe.
    Megalophrys montana (*P. montana*); Java.
  Pelobatidae (5 species of hosts, 5 (?) species of *Protoopalina*).
    Pelobates fuscus (*P. pelobatidis*); southern Europe.
    Pelobates cultripes ("*P. intestinalis*"); Montpellier, France.
    Scaphiopus bombifrons (*P. scaphiopodos*); Rocky Mountains, western North America.
    Scaphiopus hammondii (*P. hammondii*); southwestern United States, northern Mexico.
    Scaphiopus multiplicatus (*P. mexicana*); northern Mexico.
  Hylidae (5 species of hosts, 5 (?) species of *Protoopalina*).
    Hyla adelaidensis (*P. adelaidensis*); Australia.
    Hyla aurea (*P.* "*intestinalis*"); Australia.
    Hyla aurea (*P. hylarum*); Australia.
    Hyla aurea (*P. australis*); Australia.
    Hyla aurea, Cleland and Johnston (1910) report "*Opalina* sp." [doubtless a *Protoopalina*] from Queensland. Australia.
    Hyla coerulea ("*O.* sp."). Australia [of course this was probably not a true *Opalina* according to our present taxonomy, but probably a *Protoopalina*].

Anura—Continued.

Hylidae (5 species of hosts, 5 (?) species of *Protoopalina*)—Cont.

Hyla delichopsis (*P. papuensis*); Papua.

Hyla ewingii (*P.* "*intestinalis*"); Australia.

Bufonidae (6 species of hosts, 6 species of *Protoopalina*).

Bufo bufo asiaticus (*P. axonucleata*); eastern Asia.

Bufo calamita ("*P. intestinalis*").

Bufo mauritanicus ("*P. intestinalis*"); northern Africa.

Bufo melanostictus (*P. formosae*); Formosa.

Bufo peltocephalus (*P. bufonis*); Cuba.

Bufo regularis (*P. regularis*); Gold Coast, British East Africa.

Bufo regularis (*P. stevensoni*); Sudan.

Leptodactylidae (6 species of hosts, 7 species of *Protoopalina*).

Crinia signifera (*P. tenuis*); Australia.

Eleutherodactylus leptopus (*P. diplocarya*); Patagonia.

Limnodynastes dorsalis (*P. acuta*); Australia.

Limnodynastes dorsalis (*P. dorsalis*); Australia.

Limnodynastes dorsalis Cleland and Johnston (1910) report "*Opalina* sp." [doubtless a *Protoopalina*] from Queensland, Australia.

Limnodynastes peronii (*P. peronii*); Australia.

Limnodynastes peronii Cleland and Johnston (1910) report "*Opalina* sp." [doubtless a *Protoopalina*] from Queenland, Australia.

Uperoleia marmorata ("*P. intestinalis*"); Australia.

Uperoleia marmorata (*P. tenuis*); Australia.

Telmatobius jelskii (*P. longinucleata*); Ecuador.

Gastrophrynidae (3 species of hosts, 3 species of *Protoopalina*).

Gastrophryne texensis (*P. ovoidea*); southern Texas.

Gastrophryne usta (*P. xyster*); southern Mexico.

Rhinoderma darwinii (*P. rhinodermatos*); Chile.

Ranidae (7 species of hosts, 7 species and subspecies of *Protoopalina*).

Rana adspersa (*P. mossambicensis*); Mozambique.

Rana crassipes (*P. africana*); the Cameroons.

Rana esculenta (*P. intestinalis*); Europe.

Rana macrodon (*P. quadrinucleata*); Java.

Rana nigromaculata (*P. axonucleata lata*); China.

Rana nutti (*P. primordialis*); German East Africa.

Rana tigerina (*P. filiformis*); Sumatra.

Protoopalina is seen to be as well represented in the more archaic families of Anura as it is in the more modified families.

Potoopalina is known from all parts of the world except Madagascar, southern continental Asia, and eastern and northern North

America. (Fig. 239.) It is very probably present, though unreported, in southern and southeastern Asia, for it is in eastern Asia, in the East Indies and in southern Africa. Its presence in eastern and northern North America is improbable, for a rather broadly representative group of Anura from these localities have been examined. The reason for its absence from this area is not understood. *Cepedea* also is apparently absent from this region. The wide distribution of *Protoopalina* indicates that it is an old genus,

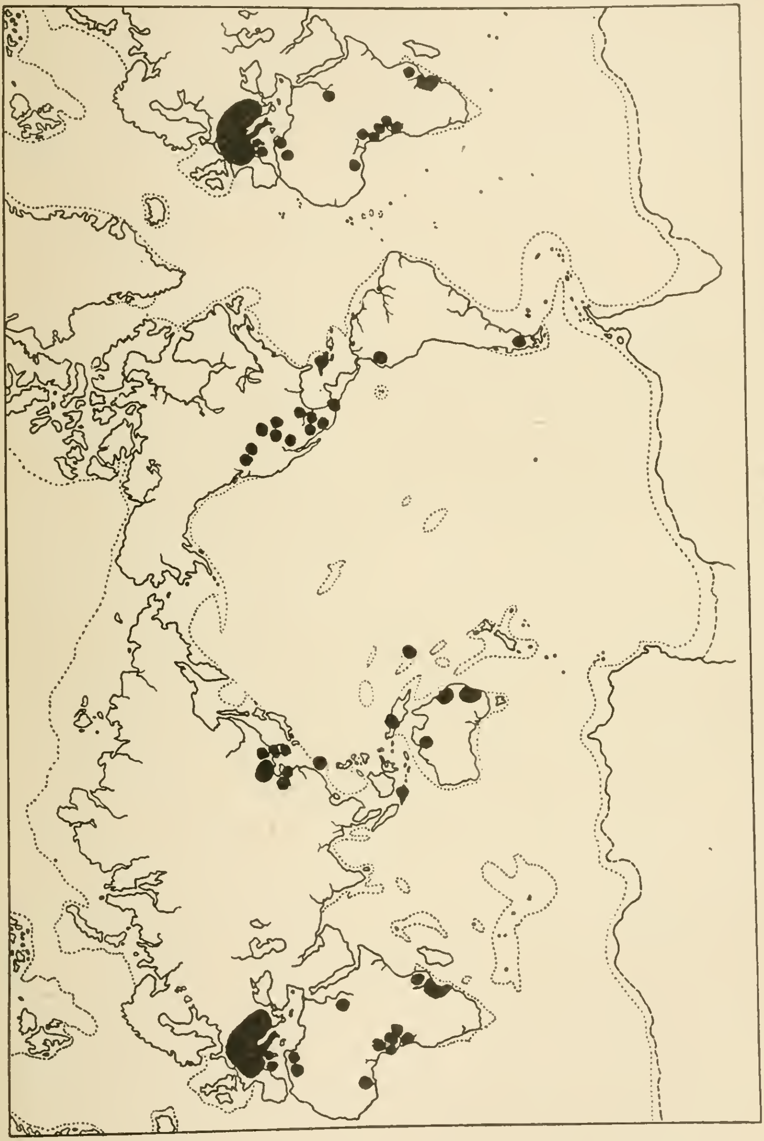

FIG. 239.—THE DISTRIBUTION OF THE GENUS PROTOOPALINA.

apparently older than the time when Australasia separated from continental Asia. It seems then pre-Cretaceous at least (figs. 233, 234). Its presence in Australasia, Africa, and South America would seemingly place its origin still a period earlier, indicating its presence as early as the Triassic in Equatoria (fig. 232), the continent including Australia, Africa, and South America. For further discussion see page 325.

There are indicated different degrees of relationship between the species of the genus *Protoopalina*, allowing us to arrange them in several subgeneric groups.[29]

Group I (fig. 240, dots).

P. diplocarya (p. 33)	in Eleutherodactylus (Leptodactylidae)	Patagonia.
P. papuensis (p. 34)	in Hyla	Papua.
P. acuta (p. 36)	in Limnodynastes (Leptodactylidae)	Australia.
P. xenopodos (p. 61)	in Xenopus (Pipidae)	Tropical Africa.

All four species have the body drawn out posteriorly into a long, slender, unciliated point. These slender, tapering Protoöpalinas have much the same form as the microgamete mother-cells of different species of *Protoopalina*, *Cepedea*, and *Opalina* and are similar in shape to the young individuals of a number of species of *Cepedea*, as, for instance, *C. dimidiata*. They are probably the most archaic group of species in the genus, though there is not definite evidence absolutely to demonstrate this. They may at least be accepted as among the most archaic species. The Australian and Papuan species are very similar to each other; so also are the Patagonian and African species. The geologic distribution of these four species, two in Australia, one in South America and one in tropical Africa, indicates a southern origin and reinforces evidence from other sources of former land connection between Australia, South America, and tropical Africa. Comparison with the charts (figs. 232 to 238) indicates origin in the equatorian continent during the Triassic or earlier. The earliest known fossil Anura are an "*Oxyglossus*"[30] (*Raninae*) from the Eocene (Gadow, 1909) or Comanchian (Schuchert, 1915 ?) of Wyoming, *Bufo serratus* from the Oligocene of Europe (Gadow, 1909), *B. gessneri* from European Miocene rocks (Gadow, 1909), and *Palaeobatrachus* (Pipidae) from the mid-Tertiary of Europe (Gadow, 1909). The geographic distribution of the characteristic parasites of the Anura apparently places the origin of the hosts at least two geologic periods earlier than their earliest known fossils. The paleontologic data are strangely scant for the Anura. The geographic distribution of the family Bufonidae will be found to be in agreement with an Equatorian origin.

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[29] All the subgeneric groups in the genera *Protoopalina, Cepedea,* and *Opalina* were demarcated before the author began the review of the geographical distribution and have not been modified in connection with this review. They therefore are based upon morphological data rather than upon distribution.

[30] Identification very doubtful.

An origin of this apparently primitive group of species of the genus *Protoöpalina* in Jurassic (fig. 233) or Cretaceous (fig. 234) times in South America or in Africa and its Madagascar-India extension; their spread from Patagonia to Australia by way of Antarctica during the later Cretaceous or the early Tertiary (fig. 236) is a complicated hypothesis which is not very probable. Any later origin

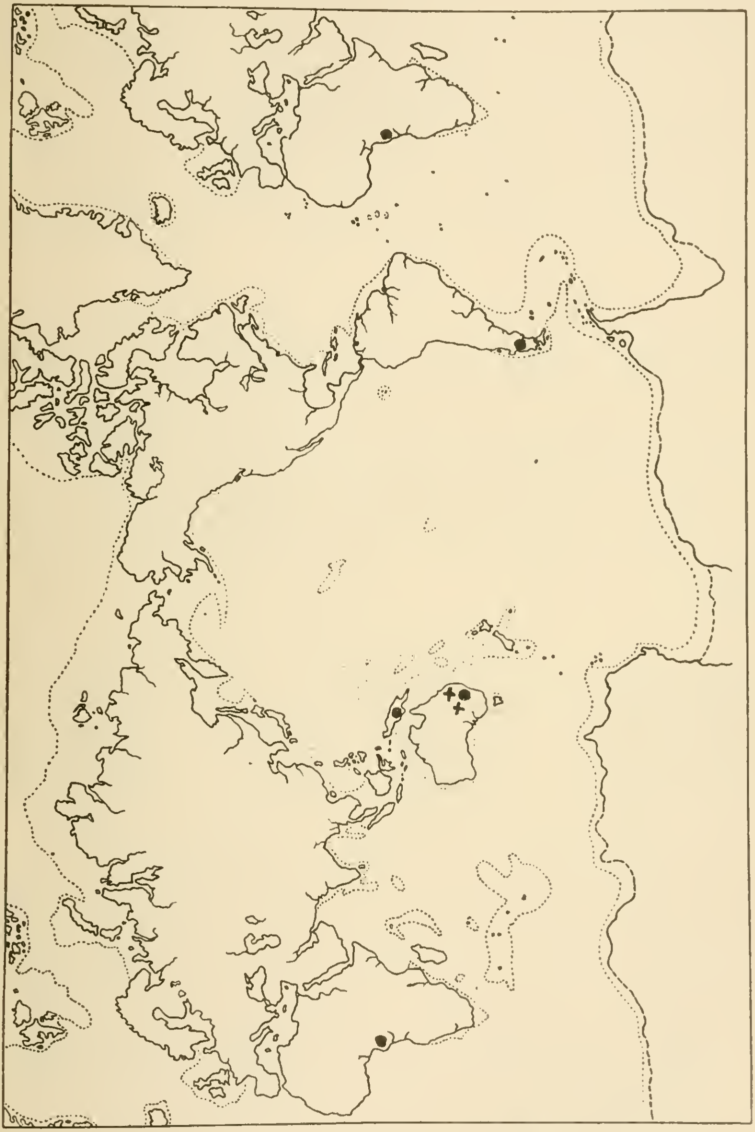

FIG. 240.—THE DISTRIBUTION OF GROUPS 1 (DOTS) AND 2 (CROSSES) OF THE GENUS PROTOOPALINA.

is barred by the present distribution of the genus *Cepedea* which is descended from *Protoöpalina.* To this we will refer later (p. 336). Compare the evidence soon to be noted that group 4 of the genus *Protoöpalina* arose in the Jurassic while Australia was connected with Asia-Malaysia but was not connected with Africa and South America (p. 317). The genus *Protoöpalina*, therefore, seems Equatorian in origin and early distribution. As this is the most archaic genus of the Opalinidea we have thus an origin of the family in Equatoria, and during the Triassic period or before.

Accepting this origin for the genus *Protoöpalina*, we may say further that in Jurassic times (fig. 233) Australia separated from Equatoria and united with Asia-Malaysia which then became populated by immigrant Protoöpalinas.

Group 2 (fig. 240, crosses).

P. dorsalis (p. 36)	**in Limnodynastes (Leptodactylidae)**	**Australia.**
P. peronii (p. 37)	**in Limnodynastes (Leptodactylidae)**	**Australia.**

These two species are considerably flattened, especially in front, and they are lance-shaped, being much less slender posteriorly than the members of the first group of species, having no naked, sharp-pointed, posterior tip. Group 2 seems nearly related to group 1. Its species were probably evolved in Australia, and at a time not indicated.

Group 3 (fig. 241).

P. caudata (p. 40)	**in Discoglossidae**	**Europe.**
P. macrocaudata (p. 49)	**in Discoglossidae**	**Eastern Asia.**
P. orientalis (p. 48)	**in Discoglossidae**	**Eastern Asia.**
P. nutti (p. 45)	**in Rana**	**Tropical Africa.**
P. stevensoni (p. 50)	**in Bufo**	**Tropical Africa.**
P. australis (p. 68)	**in Hyla**	**Australia.**

Protoöpalina caudata and *P. nutti* show both slender and stocky forms, the stocky forms having usually an abrupt, curved, round-pointed, posterior protuberance, and the slender forms being often sharp-pointed behind. *Protoöpalina macrocaudata* resembles the stocky form of *P. caudata*, while *P. orientalis* is very similar to the intermediate forms of *P. caudata*, which are neither very stocky nor very slender. *Protoöpalina stevensoni*, from the tropical African *Bufo regularis*, has a posterior process similar to that of *P. caudata*, though more spinelike, and the species is probably related to the *caudata* group. *Protoöpalina australis*, from an Australian *Hyla*, seems also to belong here.

The occurrence of members of this group in Australia, eastern Asia, Europe and tropical Africa is an indication that the group was in existence before Australia separated from Asia-Malaysia and so was pre-Cretaceous, apparently Jurassic, in origin (figs. 234, 233). They spread to Europe either during the Cretaceous (fig. 234) or in the late Tertiary (fig. 237), and to Africa in the late Tertiary

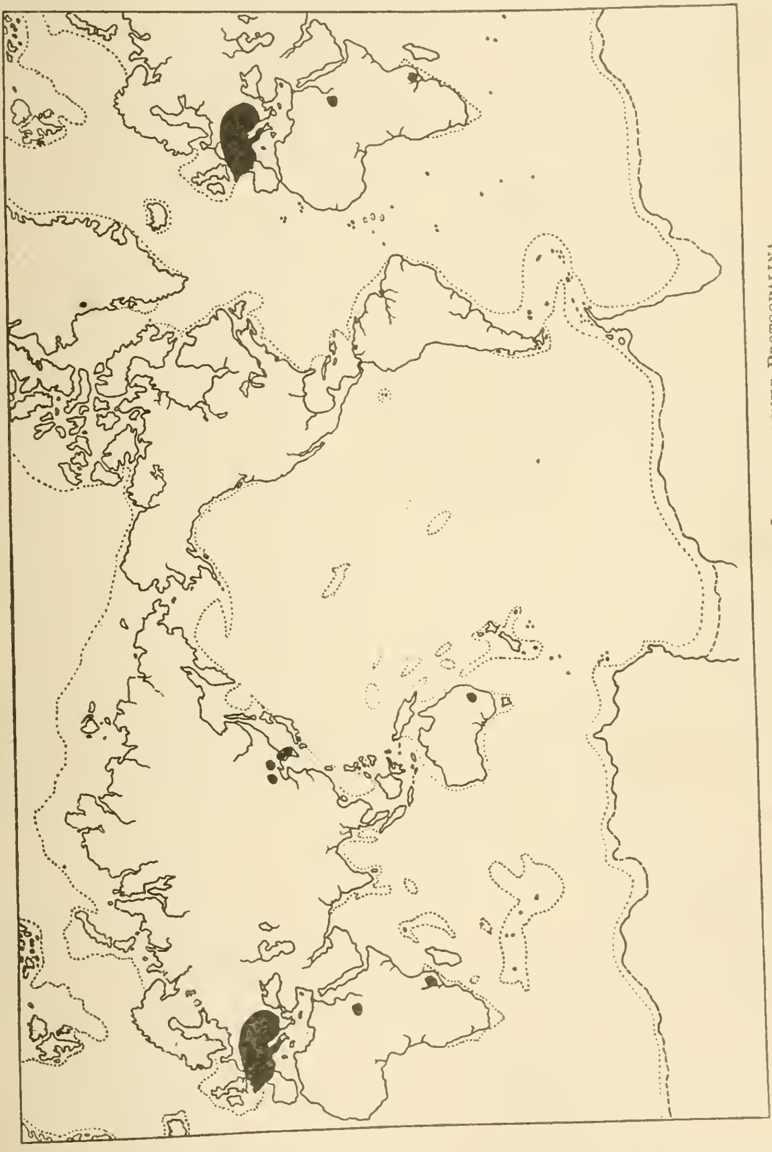

FIG. 241.—THE DISTRIBUTION OF GROUP 3 OF THE GENUS PROTOOPALINA.

(fig. 237). Their absence from South America indicates that they did not arise in Equatoria (fig. 232). Our scant records from southern Asia do not show members of this group present south of the Himalaya Mountains. Probably the spreading from the Australia-

Malaysia region was to eastern Asia, and by a route north of the Himalayas on to Europe, and into Africa from the northeast.

Group 4 (fig. 242).

P. intestinalis (p. 51)	in **Discoglossidae**	**Europe.**
P. pelobatidis (p. 38)	in **Pelobatidae**	**Europe.**
P. hylarum (p. 56)	in **Hyla**	**Australia.**

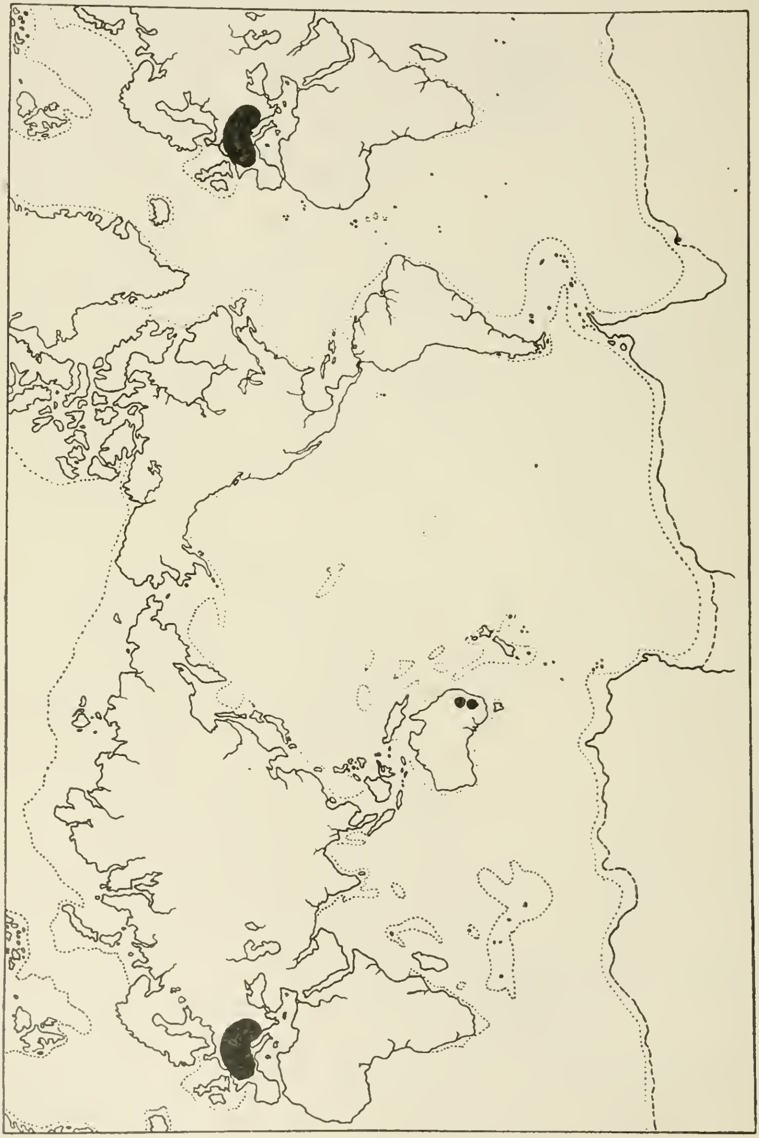

FIG. 242.—THE DISTRIBUTION OF GROUP 4 OF THE GENUS PROTOOPALINA.

Protoopalina intestinalis has been reported from hosts belonging to several other families and from other regions, but these observations need scrutiny. The presence in a Urodele, *Triturus*, is very unusual and may well be due to this newt's habit of devouring Anuran tadpoles. The infrequent occurrence in European frogs and toads may be due to temporary cross infections of these hosts by cysts of unaccustomed parasites, like many of the author's artificial cross infections of Anura with unusual species of Opalinids (Metcalf, 1909). The records from two Australian Hylas and an Australian Leptodactylid may very likely be based upon infection by similar but not identical species of *Protoopalina*, but they indicate, at any rate, the presence in Australia of one or more species of this group besides *P. hylarum.* The species of this group do not show the two forms, very stocky and very slender, nor do they have posteriorly either a sharp point or any protuberance such as that which gave the specific name to *P. caudata.* Some individuals of *P. caudata* are very similar in shape to ordinary *P. intestinalis*, but the species as a whole is quite different. Europe and Australia are the homes of the species of this group, a distribution which again argues that the group is an old one, having arisen in southeastern Asia, Malaysia, or Australasia at the time when Australia was connected with continental Asia, that is, in Jurassic times. (Figs. 232, 233, 234.) They were Jurassic in origin, rather than Triassic (fig. 232), for none of this group are known from Africa or South America. Members of this group may very likely be discovered in Asia after more thorough exploration of Asiatic Discoglossids and Pelobatids. In the absence of data from southern Asia, the available evidence indicates that the representatives of this group passed from Australia to Europe by a route north of the Himalaya Mountains, and the absence of this group from tropical Africa, so far as known, emphasizes the indication that the distribution was northern and not equatorial. It is to be regretted that our data from southern Asia are so scant.

Group 5 (fig. 243).

P. montana (p. 54)	in Pelobatidae	Java.
P. adelaidensis (p. 53)	in Hyla	Australia.

These two species approach group 4, but have quite different proportions. Their occurrence on both sides of the Bali-Lombok line is an indication of pre-Eocene origin (fig. 236).

The presence of members of this group in Australia and Java, but not in Sumatra and Asia, is in agreement with Arldt's (1907) conclusion that Java remained longer in connection with Australia than did Sumatra. The conditions seem to indicate origin in Australasia (including Java) during the Cretaceous (fig. 234). The absence of this group from South America perhaps deserves notice, since it argues slightly against the existence of a Cretaceous, trans-Pacific connection between Australasia and South America (fig. 234). Such scant negative data, however, deserves, of course, little weight.

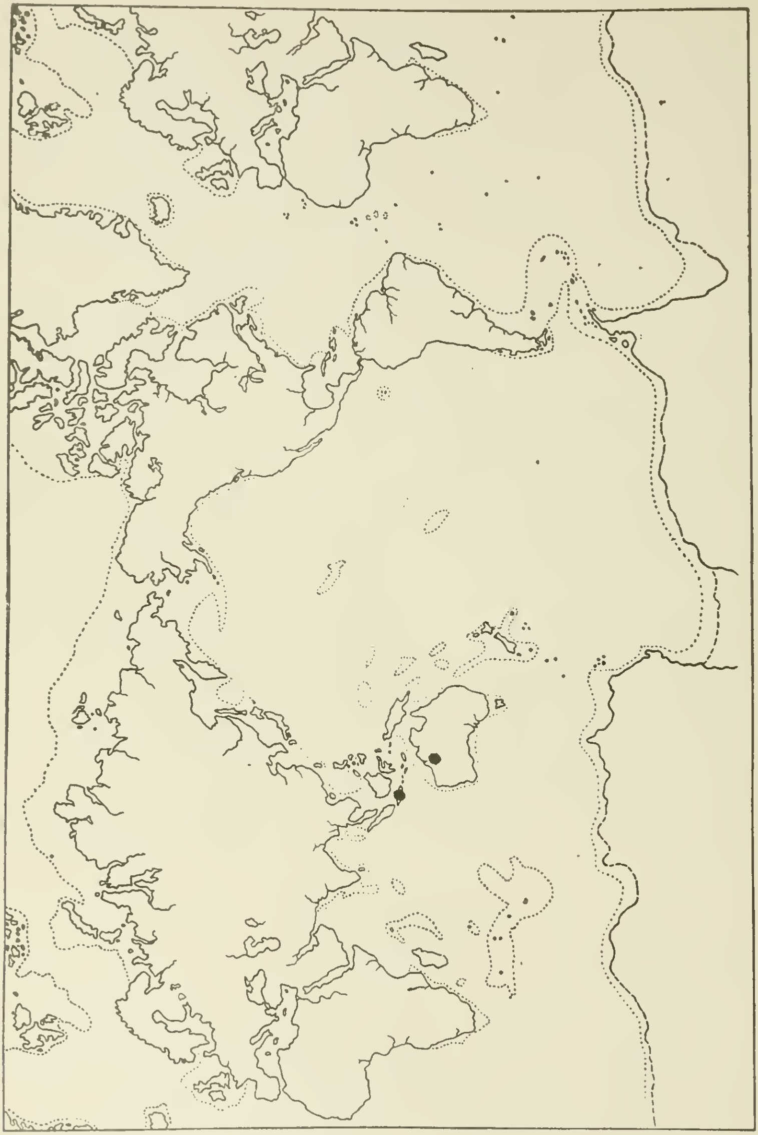

FIG. 243.—THE DISTRIBUTION OF GROUP 5 OF THE GENUS PROTOOPALINA.

Members of this group apparently did not pass eastward during the Tertiary from Australia to Patagonia by way of Antarctica (fig. 236). But this bit of negative evidence has by itself but little interest. There are, however, other indications that spreading by this route was more westward than eastward.

Group 6 (fig. 244).

P. filiformis (p. 56)	in **Rana**	East Indies.
P. tenuis (p. 58)	in **Leptodactylidae**	Australia.
P. africana (p. 60)	in **Rana**	tropical Africa.

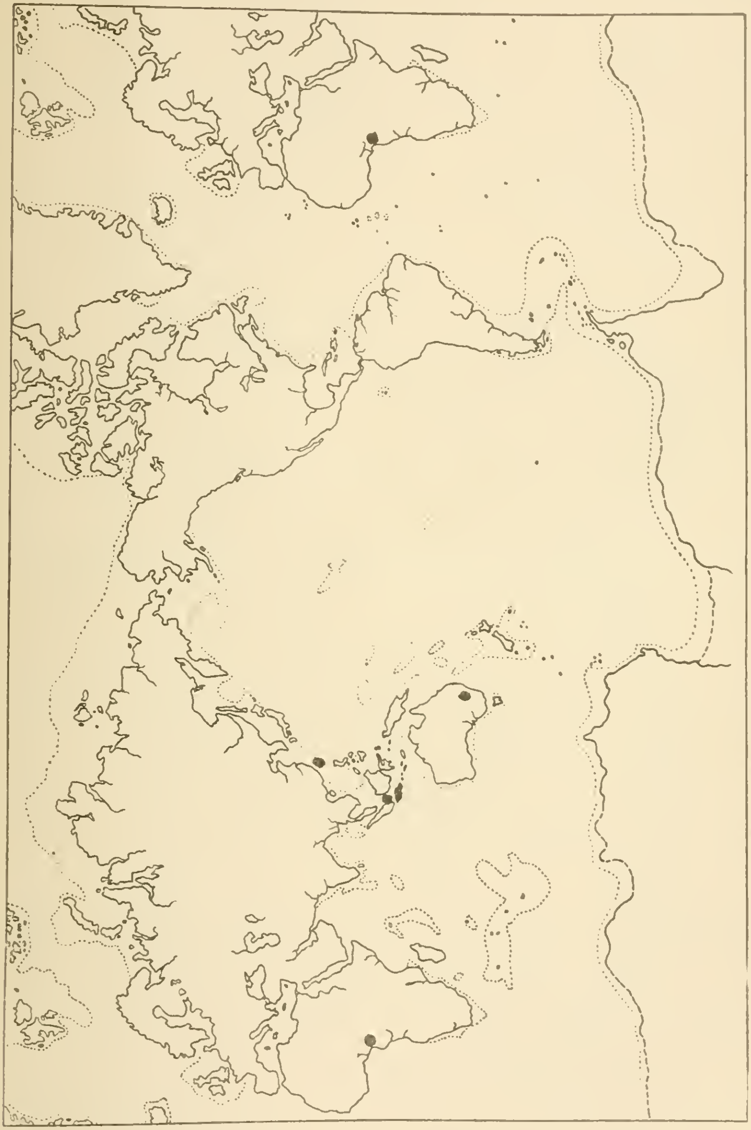

FIG. 244.—THE DISTRIBUTION OF GROUP 6 OF THE GENUS PROTOOPALINA.

These are very slender, elongated, vermiform species. The first two are very similar; the African form is somewhat less elongated and it differs from the others in being pointed behind. It may perhaps have been derived from different ancestral stock from that which gave rise to *P. filiformis* and *P. tenuis*. The occurrence of the two almost identical species, one on each side of the Bali-Lombok line, shows this group also to be an ancient one, pre-Eocene (fig. 236), although the elongated form of its species is doubtless secondary and might have led us to regard the origin of the group as more modern. The presence of one species in the northern and western Malay Islands, including Formosa, would date the origin of the group either in the Jurassic (fig. 233), and so a period earlier

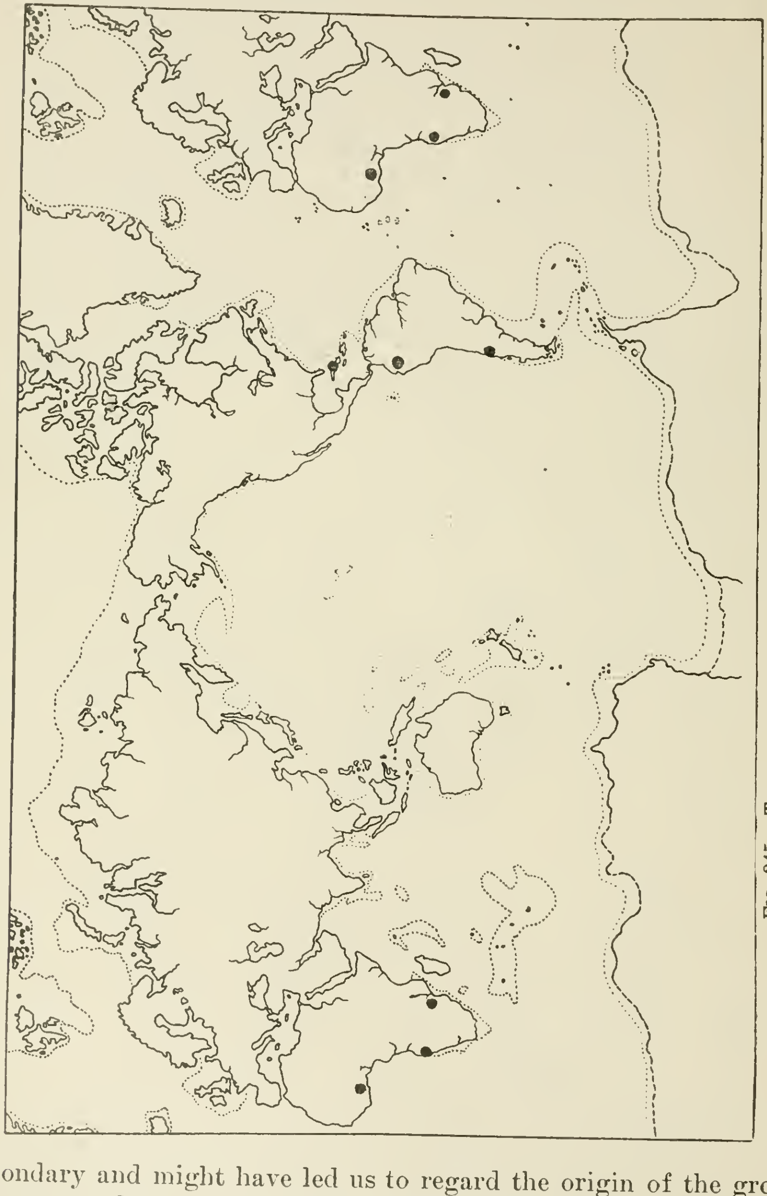

Fig. 245.—The distribution of group 7 of the genus Protoopalina.

than group 5; or in the early Cretaceous (fig. 234) while Java and Australia were connected, spreading occurring from Java to Sumatra and Formosa during the late Tertiary (fig. 237) or the Pleistocene (fig. 238).

Group 7 (fig. 245).

P. regularis (p. 70)	in Bufo	tropical Africa.
P. rhinodermatos (p. 71)	in Gastrophrynidae	South America.
P. longinucleata (p. 72)	in Leptodactylidae	South America.
P. bufonis (p. 74)	in Bufo	Cuba.

The first three species of this group have an abrupt, short, unciliated, spinelike, posterior process. *Protoopalina bufonis* lacks this spine, but the general shape of the body and the character of the nuclei in detail are very similar to the conditions in the first three species. Its relationship to this group seems probable. The occurrence of members of this group in tropical Africa and tropical America suggests origin in South Atlantis, and the period would be indicated as pre-Cretaceous or early Cretaceous (figs. 233, 234). Their absence from Madagascar is not explained. The absence from Australia would indicate a post-Triassic, that is, probably a Jurassic origin (fig. 233). The presence of *Protoopalina bufonis* in Cuba, if it be related to this group, as seems to be the case, confirms the evidence from *Zelleriella*, soon to be noted, that the West Indian lands were formerly connected with continental tropical America (fig. 237).

Group 8 (fig. 246).

P. scaphiopodos (p. 75)	in Scaphiopus (Pelobatidae)	southwestern United States.
P. hammondii (p. 76)	in Scaphiopus (Pelobatidae)	southwestern United States.
P. mexicana (p. 80)	in Scaphiopus (Pelobatidae)	northwestern Mexico.
P. mitotica (p. 77)	in Ambystoma (Urodela)	west central United States.

This very compact, sharply distinct, and highly evolved group of species have dumb-bell-shaped nuclei in a metaphase stage of mitosis. The shape of the body is rather similar to that of *P. intestinalis* and the group may be descended from immigrant ancestors from Euro-Asia, which belonged to the *intestinalis* group. The occurrence of one species in a Urodele is, of course, noteworthy. Except for this, the species of the group are confined to one American genus, *Scaphiopus*, itself similar to the Euro-Asian *Pelobates*, which bears a *Protoopalina* (*pelobatidis*) of the *intestinalis* group. Euro-Asia was probably the ancestral habitat of the Pelobatidae (fig. 224), the American genus *Scaphiopus* having become but slightly modified since its immigrant ancestor reached America, coming doubtless by way of Siberia and Alaska. In Cretaceous times (figs. 234 to 235, A) there was connection between Euro-Asia and extreme western United States, but the migration which brought to America the ancestors of *Scaphiopus* and its Protoöpalinas quite likely occurred later, for

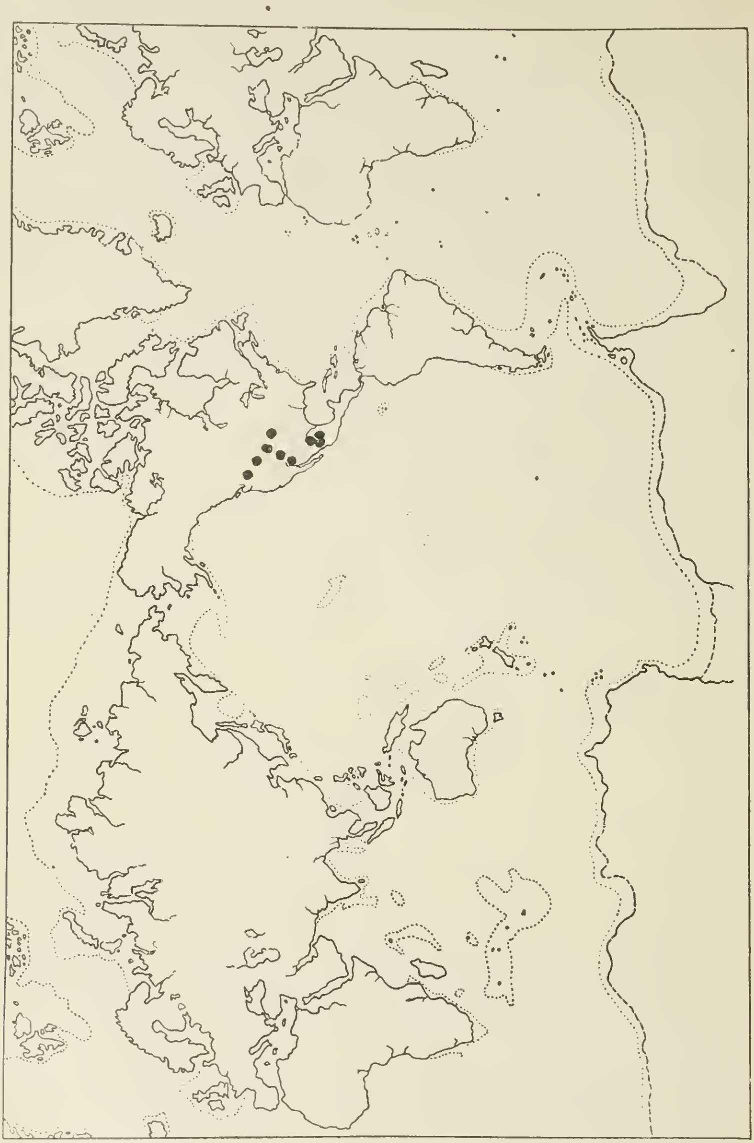

Fig. 246.—The distribution of group 8 of the genus Protoopalina.

neither host nor parasite are present in South America. The evolution of *Scaphiopus* and of its Protoöpalinas in North America was probably Tertiary or later (figs. 237, 238).

Group 9 (fig. 247).

P. formosae (p. 80)	**Bufo**	**Formosa.**
P. quadrinucleata (p. 81)	**Rana**	**Java.**
P. axonuoleata (p. 82)	**Bufo and Rana**	**Eastern Asia.**

The species of this group are not very closely similar, but they show in the three forms a progressive series in the multiplication of the nuclei, which indicates the manner of origin of the genus *Cepedea* from *Protoopalina.* We must regard these species as the most highly evolved of the Protoöpalinas, since they have passed beyond the binucleateated condition. This group of species seems at first sight to be of late Tertiary origin (fig. 237), being found in Java, Formosa, and eastern Asia, but similar forms, becoming multinucleated, must have evolved earlier, for their descendants, *Cepedea*, show a distribution which necessitates an origin as early as Jurassic times (see p. 325). Apparently transitional Protoöpalinas, with a tendency to become multinucleated, were present in the Jurassic (fig. 233) in the India-Madagascar bridge and passed during the late Tertiary to Asia-Malaysia (fig. 237). Their descendants, *Cepedea*, are in the Seychelles, Madagascar, Africa and South America. But no Cepedeas and no Protoöpalinas of Group 9 are in Australasia. The transitional forms between the two genera have persisted in Asia-Malaysia, but apparently their representatives in the Ceylon-Madagascar-Africa bridge perished during the later Cretaceous when the bridge was broken up (fig. 235), but their descendants, the Cepedeas, had already spread to Africa and South America, where they are found to-day. It seems probable that transitional species may be found in India when the Anura of this region are searched for Opalinids, for it is apparently from India that the Malaysian forms were derived.

Ungrouped Species.

We have passed four species which do not fit into any of the groups mentioned. *Protoopalina ovoidea* (p. 66) and *P. xyster* (p. 67) from two Central American species of the genus *Gastrophryne*, while not very closely similar to each other, are both flattened, *P. xyster* much so, and they indicate the probable manner of origin of the genus *Zelleriella* from *Protoopalina. Zelleriella*, as will be seen later, is in origin a southern South American genus, but the only known transitional species between the genus *Protoopalina* and the genus *Zelleriella* occur now in Central America and in species of a family not represented in Patagonia at the time *Zelleriella* was evolved there. To this interesting puzzle and its solution we will return later (p. 369).

Protoopalina saturnalis (p. 63), from the Mediterranean fish *Box boops*, is a species with two sharply contrasted forms, one very stocky, the other slender, much as in *P. caudata* and *P. nutti*, both placed in group 3, and its unciliated, tail-like, posterior tip emphasizes this resemblance, but the condition of its macrochromatin, especially in the metaphase stage of mitosis, is somewhat different from that in the *caudata* group. The life cycle and its relations to the young and the adult of the host also are different, for *P. saturnalis* reproduces sexually in the same individual adult hosts in which the adult parasites are found, and apparently the sexual

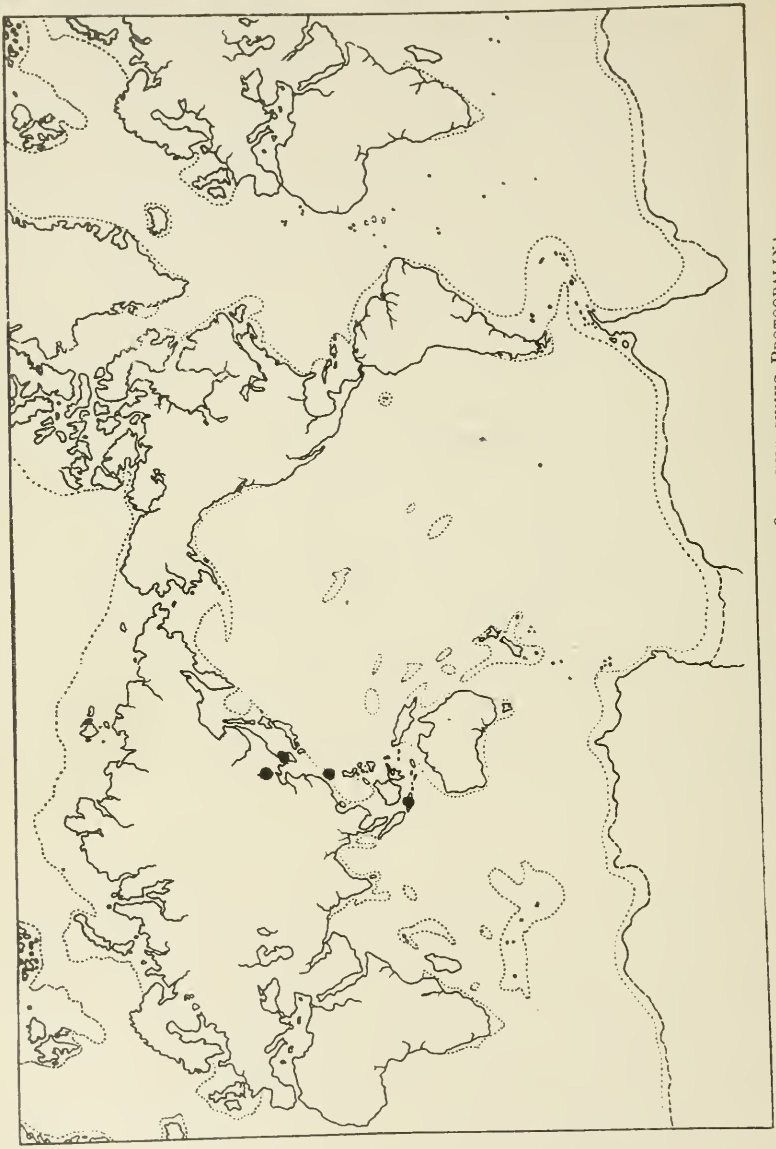

FIG. 247.—THE DISTRIBUTION OF GROUP 9 OF THE GENUS PROTOOPALINA.

phase of the life cycle of the parasite is not confined to any one season of the year. So *P. saturnalis*, while seemingly more nearly related to the *caudata* group than to any other, may hardly be placed in this group. Its occurrence in a marine fish tempts one to speculate, but there is little profit in recording such speculations. Leger and Dubosc, the discoverers of this very intesesting species, thought that because of its habitat it is probably the most primitive of the Opalinidae, but this belief seems hardly borne out by wider comparisons among the much larger number of species now known. I do not see that there are any data to indicate how *Box*, a marine fish, came to be infested with a *Protoopalina.*

Protoopalina mossambicensis (p. 73), from an East African *Rana*, seems rather widely separated from any other *Protoopalina. Protoopalina primordialis* is not sufficiently described to be included in our discussions.

The genus *Protoopalina* is so widely distributed geographically, and is known from so wide a range of hosts, that the original hosts can not readily be determined. They were probably present in the earliest Anura, for they are now known from all families of Anura. But this fact is hardly conclusive, since it is possible that some families bear adopted species rather than species which arose in the present hosts or their ancestors. This is certainly true as to the derivative genera *Zelleriella*, *Cepedea*, and *Opalina* and may be true of *Protoopalina.* We do find indications, as noted above, of the place of origin of some of the subgeneric groups of species of *Protoopalina*, and so have placed the origin of this old genus in Equatoria, the continent formed by the union of two previous land masses, Gondwanaland to the east and South Atlantis on the west, but we do not know the character of the earliest Anura in Equatoria in the Triassic or perhaps earlier period, when the Opalinidae first appeared.

Our conspectus of the occurrence of the Protoopalinas in their hosts fails to show any general limitation of closely related parasites to closely related species of hosts, though when later we view the family Opalinidae in a broader view we shall see some general host-parasite relations. *Protoopalina* is a very old genus and has had time to become adapted to diverse hosts. In this regard it is in sharp contrast to the genus *Zelleriella.* In my early studies of the Opalinidae I found it possible to infect almost any of the European species of Anura with cysts of almost any species of European Opalinid (see Metcalf, 1909), producing thus many sorts of infection not known in nature. These Opalinids in their unaccustomed hosts lived and thrived for some months. Their ultimate fate was not observed because the tadpoles could not readily be brought back alive with me from Bavaria to this country. One suspects that such unaccustomed infections may occur in nature, but fail to become permanently established because of failure in the stimuli and responses in the presexual and sexual periods. This is, however, a mere guess. The phenomena of their host distribution show that they have in the past been able to overcome the bar which hindered their entrance into and establishment in unaccustomed hosts.

The genus *Zelleriella* is reported from—

Pelobatidae (2 species of hosts, 2 species of *Zelleriella*).
- *Scaphiopus couchii* (*Z. couchii*); Texas.
- *Scaphiopus solitarius* (*Z. scaphiopodos*); North Carolina.

Hylidae (3 species of hosts, 3 species of *Zelleriella*).
- *Hyla pulchella* (*Z. hylaxena*); Paraguay.
- *Hyla septentrionalis* (*Z.* [*of Hyla septentrionalis*]); Bahama Islands.
- *Hyla venulosa* (*Z. venezuelae*); Venezuela.

Bufonidae (17 species of hosts, 14 or 15 species and subspecies of *Zelleriella*).
- *Bufo arenarum* ("*Z. antilliensis*"); Uruguay.
- *Bufo cognatus* (*Z. hirsuta*); Arizona.
- *Bufo coniferus* (*Z. opisthocarya*); Nicaragua, Costa Rica.
- *Bufo haematiticus* (*Z. bufoxena*); Nicaragua.
- *Bufo intermedius* (*Z. intermedia*); Guanajuato, Mexico.
- *Bufo lemur* (*Z. microcarya*); Porto Rico.
- *Bufo marinus* (*Z. antilliensis*); Jamaica, Bermuda.
- *Bufo melanostictus* [*?*] (*Z. macronucleata*); "Asia."
- *Bufo monxiae* (*Z. opisthocarya* [of *Bufo monxiae*]); southern Mexico.
- *Bufo orbignyi* ("*Z. antilliensis*"); Uruguay.
- *Bufo peltocephalus* (*Z.* [of *Bufo peltocephalus*]); Cuba.
- *Bufo punctatus* (*Z.* [of *Bufo punctatus*]); southern California.
- *Bufo spinulosus* (*Z.* [of *Bufo spinulosus*]); Brazil, Peru.
- *Bufo sternosignatus* (*Z.* [of *Bufo sternosignatus*]); southern Mexico.
- *Bufo typhonius* (*Z. opisthocarya* [of *Bufo typhonius*]); Panama.
- *Bufo valliceps* (*Z. intermedia cuneata*); southern Texas.
- *Bufo woodhousi* (*Z.* [*of Bufo woodhousi*]); Utah.

Leptodactylidae (17 species of hosts, 10 species (?) of *Zelleriella*).
- *Eleutherodactylus binghami* (*Z. binghami*); Peru.
- *Eleutherodactylus footei* (*Z. cusconis*); Peru.
- *Leptodactylus albilabris* (*Z. leptodactyli*); Porto Rico, southern Mexico.
- *Leptodactylus caliginosus* (*Z. leptodactyli*); southern Mexico.
- *Leptodactylus gracilis* (*Z. leptodactyli*); southern Mexico.
- *Leptodactylus microtis* (*Z leptodactyli*); Guatemala.

Leptodactylus ocellatus (*Z. brasiliensis*, "*Z. antilliensis*" (?); Brazil, Argentina, Uruguay.
Leptodactylus prognathus ("*Z. antilliensis*"); Uruguay.
Leptodactylus typhonius (*Z. magna*); Venezuela.
Limnodynastes dorsalis (*Z. binucleata*); Australia.
Limnodynastes tasmaniensis (*Z. binucleata*); Australia.
Limnomeadusa macroglossa ("*Z. antilliensis*"); Uruguay.
Paludicola bibronii (*Z. paludicolae*, "*Z. antilliensis*"); Chile, Uruguay.
Paludicola brachyops (*Z. paludicolae*); Venezuela.
Paludicola bufonina (*Z. patagoniensis*); Patagonia.
Pseudis mantidactyla ("*Z. antilliensis*"); Uruguay.
Telmatobius jelskii (*Z. telmatobii*); Ecuador, Peru.

Gastrophrynidae (6 species of hosts, 6 species and subspecies of *Zelleriella*).
Atelopus stelzneri (*Z. atelopyxena* [*stelzeri*]); Paraguay.
Atelopus varius (*Z. atelopodos*); Costa Rica.
Atelopus varius (*Z. atelopyxena*); Costa Rica.
Engystomops pustulosus (*Z.* [*engystomopsis*]); Taboga Island, Panama.
Engystomops stentor (*Z.* [*engystomopsis*]); southern Mexico,
Hypopachus variolosus (*Z. hypopacheos*); Guatemala.
Rhinoderma darwinii (*Z. darwinii*); Chile.

Dendrobatinae (2 species of hosts, 1 species of *Zelleriella*).
Dendrobates tinctorius (*Z. dendrobatidis*); Costa Rica.
Dendrobates typographus (*Z. dendrobatidis*); Costa Rica, Nicaragua.

Raninae (3 species of hosts, 3 species of *Zelleriella*).
Phyllobates trinitatis (*Z.* [*trinitatis*]); Venezuela.
Prostherapis boulengeri (*Z.* [*boulengeri*]); Colombia (South America).
Rana draytonii (*Z. ranaxena*); California.

The Zelleriellas are best represented among the Leptodactylidae and Bufonidae, the latter apparently originally an Equatorian family. They are known from four Gastrophrynidae, from two Pelobatidae, from three Hylidae, from five Ranidae, two of which are of the subfamily *Dendrobatinae* and three from the subfamily *Raninae*. *Zelleriella* is almost exclusively a western hemisphere genus, there being but one, or possibly two, species known from outside this area (fig. 248). *Zelleriella binucleata*, in *Limnodynastes dorsalis* and *L. tasmaniensis*, occurs in Australia, and *Z. macronucleata* is reported from "Asia" in *Bufo melanostictus*, which is abundant in eastern, southern, and southeastern Asia and in the East Indies. Bezzenberger (1904), who describes a number of very interesting Infusoria from "Asiatic" Anura, says of his material: "Die von mir in der Folge beschriebenen Tiere stammen alle aus einigen, für die Sammlung des zoologischen Museums in Königsberg lebend angeschafften aüszereuropäischen Anuren, deren Eingeweide (Dünn- und Dickdarm) mir von Herrn Prof. Dr. Braun zum Zweck der Untersuchung auf parasitische Infusorien gütigst zur Verfügung gestellt wurden." The presence of a *Zelleriella* in a southeastern Asian toad is so strange that one must question the Asiatic origin

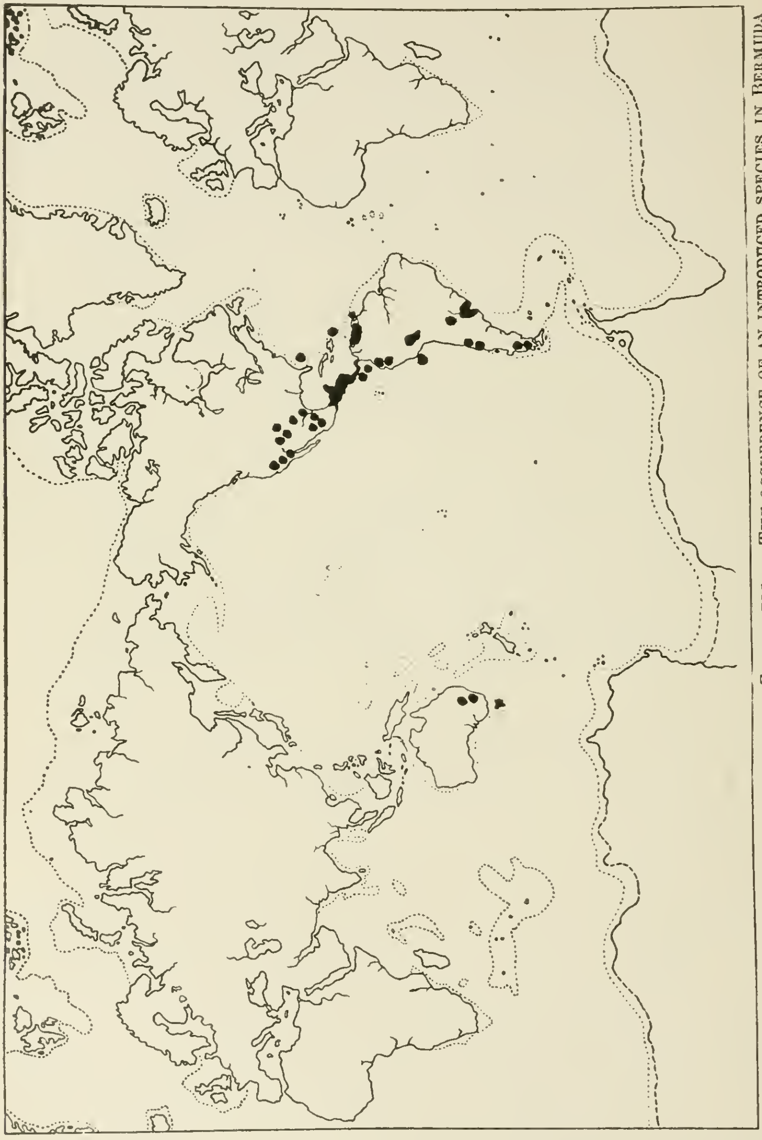

FIG. 248.—THE DISTRIBUTION OF THE GENUS ZELLERIELLA. THE OCCURRENCE OF AN INTRODUCED SPECIES IN BERMUDA IS NOT INDICATED.

of the host, even in spite of its identification as *Bufo melanostictus*. One wonders if there could have been any confusion of labels, or if the host in question might have become infected from some South American Anuran with which it may have been kept and which it may have eaten. Of course the record must stand as given, but until confirmed by further study of the parasites of *Bufo melanostictus*, one can not base conclusions as to geographical distribution upon this isolated report. I have opened 39 specimens of this toad without finding *Zelleriella*.

Zelleriella is not only a western hemisphere genus; it is chiefly South American and Central American. Two species have pushed north to California, *Z. ranaxena* from *Rana draytonii* which occurs also in Mexican California, and a form from *Bufo punctatus*, which may not be specifically distinct. Five forms have moved up into northern Mexico and southwestern United States: *Z. hirsuta*, in *Bufo cognatus; Z. intermedia*, in *Bufo intermedius; Z. intermedia cuneata*, in *Bufo valliceps; Z.* [of *Bufo woodhousi*], in *Bufo woodhousi; Z. couchii*, in *Scaphiopus couchii.* Another species, also in a *Scaphiopus* (*solitarius*), *Z. scaphiopodos*, has passed eastward along the north coast of the Gulf of Mexico to the Atlantic Seaboard. One species (perhaps also a second, *Z.* sp.?) is known from the West Indies and another occurs in the Bahamas. No account need be taken of the species in *Bufo marinus*, recently introduced into the West Indies and Bermuda. The Zelleriellas are the characteristic Opalinids of tropical and south-temperate America. It is, then, only natural that they should be abundant in two of the families abundant in this region, the Leptodactylidae and the Bufonidae. Similarly their almost complete absence from the genus *Rana* corresponds to the fact that only one Rana is known from South America. It is remarkable that only three species of the large family Hylidae are known to harbor *Zelleriella*, though the home of the Hylidae is the great forest region of tropical America; and of the three Hylas which we know bear *Zelleriella* only two belong in continental America, one being Bahaman.

The geographical distribution of *Zelleriella* outside of America is significant. *Zelleriella macronucleata* is said to occur in *Bufo melanostictus* from "Asia," but this seems so anomalous that we must leave it out of account until confirmed. Species of *Zelleriella* almost identical with this form are found in American toads. *Zelleriella binucleata* occurs in two Australian Leptodactylids, while among the American Leptodactylids occur Zelleriellas so similar that their specific distinctness from the Australian form is uncertain. Some students of geographical distribution, Gadow for example, endeavor to cast doubt upon the idea of extensive intercontinental spreading of Anura, and are inclined rather to believe, in some cases, that our Anuran families and subfamilies are not formed in accordance with true genetic relationships, and that convergent independent evolution accounts for the resemblance. Gadow (1909, p. 71) writes:

> It is one of the most difficult tasks to decide in cases of great resemblance of groups of animals between their being due to direct affinity, or to heterogeneous convergence, or parallel development. * * * the startling view that Madagascar and South America have received part of their faunas from the famous Antarctica. * * * The *Dendrobatinae* (*Mantella* in Madagascar, the others in South America) are decidedly not a natural group, but an instance of very recent convergence.

Writing of the Pipidae, Gadow says (1909, p. 145):

> We conclude now that all these four genera [*Pipa*, *Xenopus*, *Hymenochirus*, and the European mid-Tertiary *Palaeobatrachus*] belong to one group with a distribution formerly much wider than Africa and part of South America.

Zelleriella apparently definitely refutes the hypothesis of convergence, so far as the Australian and South American Leptodactylidae are concerned. It would perhaps be conceivable, though difficult to believe, that the Australian Leptodactylids may have evolved independently of the South American forms now classed in this family. But it is hardly conceivable that almost identical internal parasites were evolved also independently in the two groups of hosts. *Zelleriella* is a very compact genus morphologically, so compact that subdivision into valid species is difficult. The Australian *Z. binucleata* and some American Zelleriellas are especially similar. There seems no escape from the conclusion that the Leptodactylids of America and Australia, and their parasites as well, arose in some one region and spread to their present localities. The evidence for an Antarctic land connection between South America and Australia is greatly strengthened by the data *Zelleriella* and the Leptodactylidae present. Indeed the evidence seems conclusive. We should remember here the fact that *Protoopalina diplocarya* from Patagonia and *P. acuta* from Australia, both parasitic in Leptodactylids, also *P. papuensis* from New Guinea, parasitic in a *Hyla*, are forms very closely related to one another. The Leptodactylidae are developed in greatest variety and number in South America and probably arose in Patagonia or lands connected with Patagonia. Their *Zelleriella* parasites apparently arose in the same region, being derived from the Protoöpalinas by the flattening of the body. *Protoopalina xyster* of Central America is a partially flattened form which suggests the sort of intermediate stage through which the ancestors of the *Zelleriellas* may have passed.

The same line of reasoning does not hold as to *Bufo* and *Zelleriella* and a land connection between South America and Asia, for the report of the single infection of *Zelleriella* in an Asiatic toad is so remarkable and so indefinite that unless it be confirmed it can hardly be accepted for discussion of distribution.

It seems in agreement with the data at present known to suppose that a great continental mass existed in the Southern Hemisphere up into Miocene times (fig. 236), and that upon this continent, including Australasia and southern South America, there were Leptodactylids which had *Zelleriella* parasitic in them. *Bufo* was not in this Antarctic fauna. At this time there was across south-central South America a shallow sea connecting the Atlantic and Pacific Oceans, which, from the evidence of the Andean strata, seems to have persisted to a time as late as the last of the Miocene or apparently the early Pliocene. But earlier than this, during the Miocene, the region to the south of Patagonia subsided and Patagonia became separated from Graham's Land, the land route from Patagonia to Australia being thus closed. The separation of Patagonia from Australia occurred before Patagonia became definitively united to tropical America. After the establishment of land connection between the northern and southern portions of South America, the toads passed southward to occupy Patagonia, the Leptodactylids at the same time passing northward into northern South America, Central America, and the West Indies. When the toads and the Leptodactylids met, the latter gave *Zelleriella* to the toads. We will discuss later the evidence as to the origin and spreading of *Bufo* and the Bufonidae.

Zelleriella, now overwhelmingly tropical American, probably arose in Patagonia-Argentina and we may suppose that it arose only a short time before Australia separated from Patagonia, since we know as yet but one species of *Zelleriella* from Australia. When Patagonia united with tropical America, the Leptodactylidae and their Zelleriellas passed throughout tropical America. There may have been some Bufonidae with them, but, if so, of other genera than *Bufo*. Bufos, and other genera of Bufonidae, were doubtless already present in tropical America, for an Africa-America trans-Atlantic bridge had probably brought Bufonidae from Africa long before the Pliocene (p. 299). When they met the Leptodactylids, the Bufonidae adopted the Zelleriellas, but did not in Central America give their own Cepedeas to the Leptodactylids.

A land connection between the West Indies, the Bahamas, and the continent apparently must have been in existence after the northern and southern portions of South America were united, for, as the Leptodactylids came north they were able to pass to the Antillean region, carrying their Zelleriellas with them. The immigrant toads now living in the West Indies bear adopted Zelleriellas, very likely adopted before their immigration from the continent. The *Dendrobatinae* are also represented in the Antilles as well as upon the continent, and a *Hyla* bearing *Zelleriella* is in the Bahamas. We have therefore here five parallel lines of zoögeographic evidence of connection between the West Indies and the Bahamas on the one hand and the American continent on the other hand. The species of *Zelleriella* are all so much alike that subgeneric groups of species are not to be recognized. In consequence we can not determine whether the Zelleriellas of the Antillean and Bahaman toads, Leptodactylids and Hylas, are more nearly related to the continental species or to other West Indian species. The contour of the floor of the Caribbean Sea and of the Gulf of Mexico indicates that the connection of the West Indian lands was with Central America by probably two ridges (fig. 237), one from Cuba to Yucatan and another from Jamaica to Honduras (Vaughan, 1919). Arldt places this connection in the Miocene, but Vaughan's more recent study places it in the Pliocene. The southern tip of the Florida peninsula was probably connected with West Indian lands for a brief period, but the distribution of Anura and Opalinids seems not significant in relation to this connection.

It is noteworthy that the Leptodactylidae did not pass northward beyond Central America, except for two species that have penetrated into Texas. They are found in portions of South America seemingly no better suited to them than are portions of North America. It looks as if the desert region of northern Mexico and southwestern United States held back these moist-skinned forms, for they, like the Ranas, are not resistant to drouth. If so, the desert character of the area mentioned must be of long standing, having existed at least since the Pliocene, as noted in connection with discussion of the distribution of the Ranas (p. 374). Observe also that the Pelobatidae in the north do not spread southward past the Mexican desert (fig. 224). Some other groups, approaching from the south, find in northern Mexico a barrier to their further migration: Gastrophrynidae (fig. 229), only one genus (3 species) being known north of Mexico; *Dendrobatinae* (fig. 231); the archaic Urodele family Coeciliidae (fig. 256, p. 382); and two equatorial families of freshwater fishes, Cichlidae (fig. 257) and Characiindae (fig. 258). We thus see the probable reason why *Zelleriella* is not abundant in North America.

The evidence from *Zelleriella*, then, indicates that the Leptodactylids are an Antarctic family and that *Bufo* had a more northern origin (p. 362). *Zelleriella* itself is an Antarctic genus. *Zelleriella* is older than the time when northern and southern South America became joined, and probably more recent than the time when Australia separated from Papuasia, and of course then is more modern than the time of separation of Australasia from Malaysia. This would place the origin of *Zelleriella* apparently in the Miocene, or but little earlier.

Species of the genus *Cepedea* are reported from—

Discoglossidae (1 species of host, 1 species of *Cepedea*).
- *Alytes obstetricans* (*C. minor*); central France.

Pelobatidae (2 species of host, 2 (?) species of *Cepedea*).
- *Scaphiopus albus* (*C. floridensis*); Key West, Florida.
- *Scaphiopus solitarius* (*C. ? flava*); locality doubtful, probably eastern North America.

Hylidae (5 species of host, 5 species and subspecies of *Cepedea*).
- *Hyla albomarginata* (*C. multiformis*); Bonito, Brazil; Nicaragua.
- *Hyla baudinii* (*C. baudinii*); Guatemala; Cordova, Mexico.
- *Hyla nassica* (*C. dimidiata paraguensis*); Paraguay.
- *Hyla versicolor chrysoscelis* (*C.* sp.?); Texas.
- *Phyllomedusa lemur* (*C. globosa*); Costa Rica.

Bufonidae (9 species and subspecies of host, 8 species of *Cepedea*).
- *Bufo bufo* (*C. dimidiata*); Europe, Asia (?).
- *Bufo formosus* (*C. fujiensis*): Japan.
- *Bufo gargarizans* (*C. buergeri sinensis*); Hong Kong, China.
- *Bufo haematiticus* (*C. dolichosoma*); Costa Rica, Nicaragua.
- *Bufo jerboa* (*C. borneonensis*); Borneo.
- *Bufo latifrons* (*C. magna*); the Cameroons, West Africa.
- *Bufo lentiginosus* (*C. obovoidea*); Florida.
- *Bufo melanostictus* (*C. formosae*); Hong Kong, China; Formosa.
- *Bufo melanostictus* (*C. pulchra javanensis*); Java.
- *Bufo viridis* (*C. dimidiata*); Europe, Asia (?).

Gastrophrynidae (2 species of host, 2 species of *Cepedea*).
- *Kalvula pulchra* (*C. pulchra*); Cochin China.
- *Phrynomantis bifasciata* (*C. phrynomantidis*); British East Africa.

Ranidae (20 species and subspecies of hosts, 18 species and subspecies of *Cepedea*).
- *Hyperolius marmoratus* (*C. madagascariensis* [of *Hyperolius*]); west Africa.
- *Megalixalus madagascariensis* (*C. madagascariensis*); Madagascar.
- *Megalixalus seychellensis* (*C. seychellensis*); Seychelles Islands.
- *Oxyglossus lima* (*C. spinifera*); Java.
- *Polypedates buergeri* (*C. buergeri*); Japan.
- *Polypedates leucomystax* (*C. segmentata*); Cochin China, Java, Formosa.
- *Polypedates schlegelii* (*C. multiformis* [of *Polypedates schlegelii*]); Japan.
- *Rana cantabrigensis* (*C. cantabrigensis*); north-western North America.

Rana cantabrigensis latiremis (*C. cantabrigensis*); Alaska, western Canada.
Rana catesbeiana (*C. dimidiata hawaiensis*); Hawaiian Islands (introduced).
Rana chrysoprasina (*C. occidentalis*); Nicaragua.
Rana esculenta (*C. dimidiata*); Europe, Asia.
Rana esculenta hispanica (*C. hispanica*); Spain.
Rana esculenta ridibunda (*C. saharana*); Algiers.
Rana limnocharis (*C. longa*); Japan, Formosa.
Rana nigromaculata (*C. dimidiata orientalis*); Japan.
Rana pipiens (*C. mexicana*); north-eastern Mexico.
Rana rugosa (*C. pulchra japonica*); Japan.
Rana temporaria (*C. dimidiata*); Switzerland.
Rana tigerina (*C. ophis*); Formosa; Billiton Island, near Sumatra.

The Cepedeas are known from but one species each of the families Discoglossidae and Pelobatidae; have been found in five species of Hylidae; are well represented in the Bufonidae; are poorly represented in the Gastrophrynidae (2 species only); are not known from the Leptodactylidae and *Dendrobatinae;* and are abundantly represented in the *Raninae.*

The geographic distribution of the Cepedeas may similarly be summarized (fig. 249). From Europe four species are known (in *Discoglossus*, *Bufo* [2], *Rana*), three of which extend to temperate Asia; from eastern Asia, including Japan and Formosa, thirteen species and subspecies are known (from *Bufo* [6], *Kaloula* [1], and Ranidae [7] [31]), of which one extends into Formosa and two extend into the East Indies; from Australasia none are known; from northern Africa one is reported in *Rana esculenta ridibunda;* from western Africa two species are known (from *Bufo* and from *Hyperolius*, a Ranid); from southeastern Africa we know one species (from *Phrynomantis*, a Gastrophrynid); from Madagascar and the Seychelles Islands two species are reported (from *Megalixalus*, a Ranid genus); from central South America one species is known (in a *Hyla*), another is reported from Brazil and from Central America (in a *Hyla*); from tropical Central America we know five species (from Hylidae [3], *Bufo* and *Rana*) one of which extends into Brazil; from southwestern North America, including northern Mexico, which belongs to the same geographical region, we know two species (from a Hylid and from a *Rana*); northwestern North America gives us two species (in two Ranas); and from southeastern North America we know two species (in a Pelobatid and a *Bufo*); from northeastern North America no Cepedeas are known.

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[31] These numbers do not total 12, for one of these species of *Cepedea* is found in both *Bufo* and *Rana*.

The genus *Cepedea* is thus seen to be cosmopolitan, except that it is not known from Australasia or northeastern North America. The absence of *Cepedea* from northeastern North America, like the absence of *Protoopalina* from the same region, is unexplained. Suitable hosts are present in abundance. The region is no colder than regions from which both genera are known. The absence of *Cepedea* from Australia would seem at first thought to be an indication that

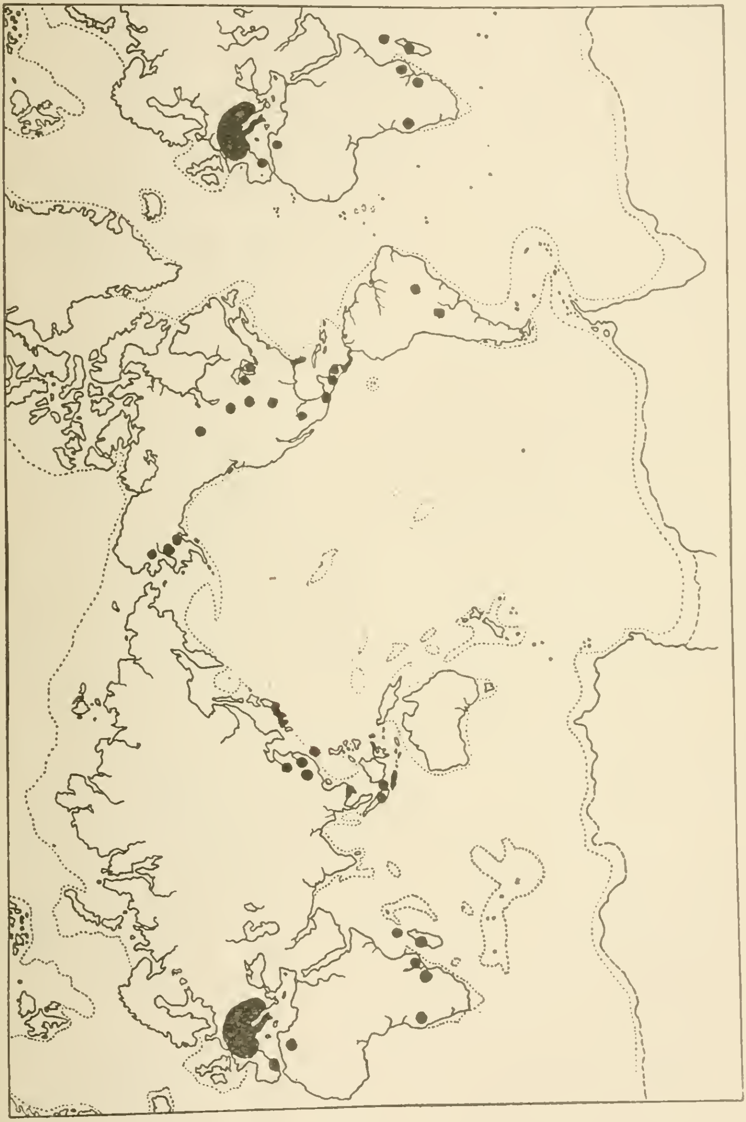

Fig. 249.—The distribution of the genus Cepedea.

the genus arose since early Cretaceous times, when Australasia separated from Malaysia (fig. 234), but study of the distribution of the several groups of species in the genus will give different indication, namely, that *Cepedea* evolved probably during the Jurassic from group 9 of the species of *Protoopalina*, or from similar forms, and that its place of origin was probably India or some portion of the India-Ceylon-Madagascar-Africa bridge (p. 346). The absence of both *Protoopalina* and *Cepedea* from northeastern North America shows that there may be other factors than mere land connection which influence the distribution of Opalinidae. Two-thirds of the known species of *Cepedea* are from the Eastern Hemisphere, a fact which is some indication that the genus arose in the east. This seems the more true, since we have so scant data from southern Asia. Probably the list of eastern species would be considerably increased if we knew the southern Asian forms. Another indication of Asiatic origin of *Cepedea* is the fact that the four species (1 *Cepedea*, 3 *Protoopalinae*) which intergrade between *Protoopalina* and *Cepedea*, are all Asian or Malaysian (see the second paragraph following). The evolution of the genus *Cepedea* also culminates in the east, in *C. longa* and *C. segmentata* from eastern Asia and Malaysia.

The absence of broad Opalinas from New England and the absence of narrow Opalinas from European Ranas is of interest. *Rana temporaria* of Europe (which bears the broad *Opalina ranarum*) and *R. sylvatica* of New England (which carries the narrow *Opalina virguloidea*) are very close relatives. The ancestor of one or the other of them probably crossed between the two hemispheres by way of the Greenland-Iceland North Atlantic bridge. But whichever one so crossed did not carry its *Opalina* with it. It is possible, this migration took place from New England to Europe before the American *Rana sylvatica* had met a Hyla and adopted its narrow *Opalina*. (The narrow *Opalinae* probably evolved in the Hylids.) *Hyla* reached North America probably during the latter half of the Pliocene. If the migration of the ancestor of *Rana sylvatica* to Europe occurred before this, it would have had no narrow *Opalina* to carry with it. This suggestion would date the Greenland-Iceland bridge as early as the Middle Pliocene or earlier.

But we can recognize several groups of related species within the genus *Cepedea* and this allows us to analyze the distribution still further. The subgeneric affinities of some species are doubtful, but there are other species which so resemble one another as to form fairly well demarcated groups.

Division 1.

C. lanceolata (p. 137)	in Rana	"Asia."

This species is not similar to any other *Cepedea* (fig. 102, p. 137), no other species having so slenderly tapering a body posteriorly, or having so few nuclei. Its form is much like that of a microgametocyte. It resembles very young *Cepedea dimidiata* in shape and in its small number of nuclei. In body form and number of nuclei this *Cepedea* resembles *Protoopalina quadrinucleata*, but the nuclei of the former are *Cepedea* nuclei and the nuclei of the latter are *Protoopalina* nuclei. *Cepedea lanceolata* seems to be a transitional form between the genera *Protoopalina* and *Cepedea* and is probably the most archaic of the known Cepedeas. The highly evolved species of *Protoopalina* (group 9), which approach the genus *Cepedea*, and the most archaic species of *Cepedea* (*lanceolata*) occur in the Malay Islands and in "Asia." This is some indication that the genus *Cepedea* arose in the general region of Malaysia or eastern Asia, but there are other indications of its origin in India in post-Triassic times, after Australia had broken away from Equatoria (figs. 233 and 234). This suggestion of eastern origin is somewhat emphasized by the fact, already mentioned, that two-thirds of the known species in the genus are now living in the eastern hemisphere. The absence from Australia of transitional species between the two genera and of all species of *Cepedea* is an indication that the genus *Cepedea* evolved since Australia separated from Equatoria. It seems clearly to be a more modern genus than *Protoopalina*.

Division 2 (fig. 250).

C. dimidiata (p. 139)	in Rana and Bufo	Europe, eastern Asia.
C. dimidiata hawaiiensis (p. 143)	in Rana	Hawaii, introduced.
C. dimidiata orientalis (p. 141)	in Rana	Japan.
C. dimidiata [paraguensis] (p. 142).	in Hyla	Paraguay.
C. saharana (p. 144)	in Rana	northern Africa.
C. buergeri (p. 145)	in Polypedates (Raninae)	Japan.
C. buergeri sinensis (p. 146)	in Bufo	southern China.
C. minor (p. 147)	in Alytes (Discoglossidae)	France.

All but the last named of the species of this division have individuals of two forms, stocky and slender, and in all regards they are considerably alike. The affinities of *Cepedea minor* are doubtful and it should probably be left out of account in the discussion of distribution. Excepting the Paraguayan form, which is classed as a subspecies of *C. dimidiata* and the Hawaiian form which must have been introduced by man, the other members of the group all belong to the one zoögeographical region including Asia north of the Himalayas, Europe, and northern Africa, the latter of which zoögeographically may be considered almost a part of Europe.

Cepedea dimidiata [*paraguensis*] is a puzzle, for it is found in southern South America in a *Hyla.* Only one other *Cepedea* is known from South America, this in a Brazilian *Hyla.* The other four Hylidae which are known to bear *Cepedea* live in Central America and Texas, and their Cepedeas, or their ancestors, probably were brought from Asia by way of Alaska and western North America, or may possibly have come from Africa to tropical America. Only three Anura from tropical Africa are now known to bear *Cepedea;* these are a *Bufo*, a *Phrynomantis* (Gastrophrynidae) and a *Hyperolius* (Ranidae). We shall see that *Bufo* seemingly entered

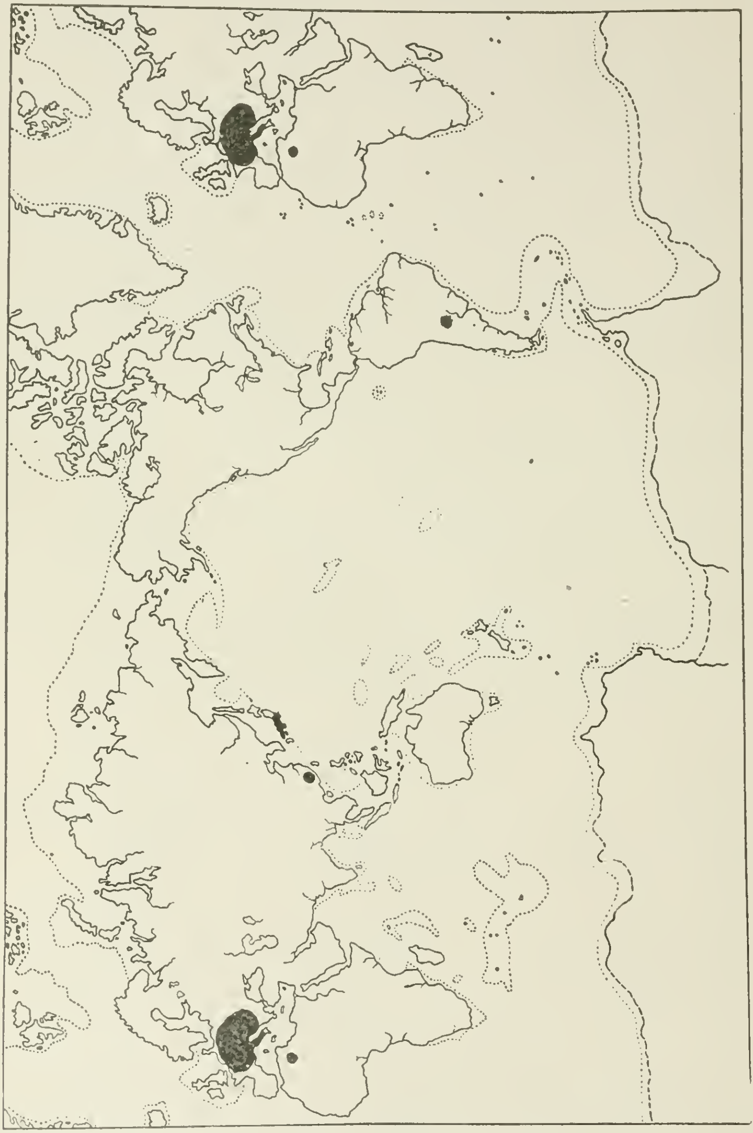

FIG. 250.—THE DISTRIBUTION OF DIVISION 2 OF THE GENUS CEPEDEA.

Africa from the north at a period after Africa had broken its connection with South America (p. 363). The genera *Hyperolius* and *Phrynomantis* have not reached tropical America. Apparently no *Raninae* have entered South America from Africa, but Gastrophrynidae seem to have come by this route and may have brought *Cepedea. Pipa*, or rather its ancestor, may have been an immigrant from Africa, for its known relatives are two African genera, *Xenopus* and *Hymenochirus*, and *Palaeobatrachus*, from mid-Tertiary deposits in central Europe. No Opalinidae are known from *Pipa.* The ancestors of the *Dendrobatinae* of South America probably came from Africa, but neither American nor African members of this subfamily are known now to carry *Cepedea*, *Bufo*, being absent from Madagascar, probably was not in continental Africa until after Africa lost connection with South America (fig. 235, p. 302). The Gastrophrynidae are, therefore, the most probable hosts for African Cepedeas entering South America. The Cepedeas have been brought to America in hosts which did not belong to the Hylidae which is American and Australian and not Euro-Asian or African. *Cepedea dimidiata* [*paraguensis*] shows no close similarity to any other American species, or to any known African species, and I do not see that we have any light to help us understand the presence in southern South America of this apparent near relative of the Euro-Asian Cepedeas of this second group. There are no known members of this group in tropical Africa or in North America. Further knowledge of the *Cepedea* faunas may sometime solve this puzzle for us, but we find no solution in the data now known.

Division 3.

C. spinifera..................in Oxyglossus (Raninae)..................Java.

This species, which bears an abrupt, unciliated, spinelike, posterior process, seems not to be very closely related to any other known species of *Cepedea*, its posterior spine and long cilia being distinctive, *Oxyglossus*, its host, is known from India and the East Indies. A fossil form assigned to this genus is said to occur in the "Eocene" or "Comanchian" of Wyoming.[32] *Cepedea spinifera* may have arisen in Java in the Pleistocene after Java became finally isolated.

Division 4, A (fig. 251, dots).

C. phrynomantidis (p.148).in Phrynomantis (Gastrophryninae)...............East Africa.
C. madagascariensis (p.149)..in Megalixalus (Raninae).Madagascar.
C. madagascariensis [of. Hyperolius] (p. 150). in Hyperolius (Raninae). Western Tropical Africa.
C. magna (p. 150).......in Bufo.....................Western Tropical Africa.
C. obovoidea (p. 151)....in Bufo...................Florida.

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[32] Gadow (1909), "Eocene"; Schuchert (1915?), "Comanchian," but the identification as *Oxyglossus* must be regarded as very doubtful.

This group of species is set apart from other species by the great development of the axial excretory vacuole. All but *C. obovoidea* are shaped much like the slender forms of *C. dimidiata*. *Cepedea abovoidea* is flattened in front and, in consequence, is broader in this part of the body. Its flattened anterior end separates it a little from the other species, to which, however, it seems related. So far as available evidence goes, it was probably derived from an African member of this group, which migrated to America. Its presence in Florida might seem to suggest a former direct connection between Florida and

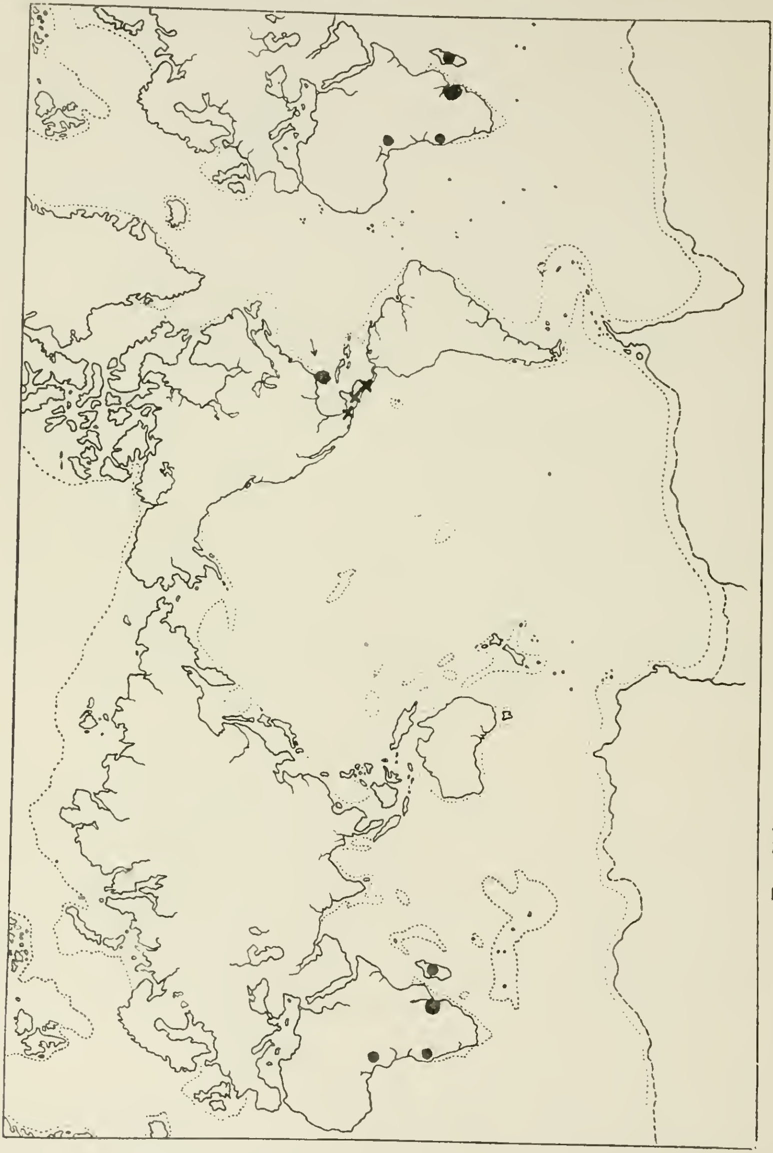

FIG. 251.—THE DISTRIBUTION OF DIVISION 4 OF THE GENUS CEPEDEA.

the West Indian lands, a connection which probably did once exist for a brief period. To reach the West Indian lands it apparently had to pass by way of Central America, and it might about as readily have passed from Central America to Florida along the Gulf coast as across the Caribbean land bridge to Cuba and then to Florida (fig. 237, p. 305). Connection of southernmost Florida with Cuba in Miocene or Pliocene times has been suggested. Connection of northeastern South America with the Lesser Antilles, the eastern arch of the West Indian Islands, has also been suggested for pre-Miocene or early Miocene times, but we do not have clear indication of union of South America with the Greater Antilles by this eastern route. The Africa-South America connection was probably broken by this time. Von Ihering (1900) places the interruption of the Africa-South America connection not later than the early Tertiary; Eigenmann (1909) places it before the Tertiary; so also do Arldt (1907), Haug (1907–1911), and Schuchert (1915?).

One member of this division 4, *A*, is parasitic in *Phrynomantis*, a Gastrophrynid. The distribution of this family of Anura, as we shall later see, emphasizes the belief in a direct land connection between western Africa and tropical America (fig. 229, p. 293), making more credible the origin of the Florida *Cepedea* from an immigrant from Africa.

Division 4, B (fig. 251, crosses).

C. globosa (p. 153) in **Phyllomedusa** (**Hylinae**)	Central America.
C. baudinii (p. 154) in **Hyla**	Central America.

These two species have greatly developed, axial, excretory vacuoles like those in the members of division 4, *A*, but in shape of body they are quite different. Their presence in Central America emphasizes the suggestion already made that the Florida representatives of these highly vacuolated Cepedeas probably came by way of Central America. These two species may well have evolved from some member of division 4, *A*.

Division 4 as a whole, now represented in both tropical Africa and tropical America, apparently evolved in South Atlantis, that is, the Africa-South America land mass, during the Jurassic (fig. 233) or the early Tertiary (fig. 234), after Australia broke away from Equatoria.

Division 5 (fig. 252).

C. pulchra (p. 155) in **Kaloula** (**Gastrophryninae**)	Cochin China.
C. pulchra japonica (p. 156) in **Rana**	Japan.
C. pulchra javensis (p. 156) in **Bufo**	Java.
C. occidentalis (p. 157) in **Rana**	Tropical Central America.
C. floridensis (p. 158) in **Scaphiopus** (**Pelobatidae**)	Key West, Florida.

The three forms from eastern Asia and Java it seems best to place in one species. They are somewhat flattened. The two western species are more flattened, especially in front, and approach the condition of the genus *Opalina*. None of the forms shows a greatly developed, axial system of vacuoles. The most flattened of the five, and so probably the most modified, is *C. floridensis*, whose occurrence at Key West, Florida, in a species of *Scaphiopus* (*albus*)[33] known only from the Florida keys, is of interest. This is the only

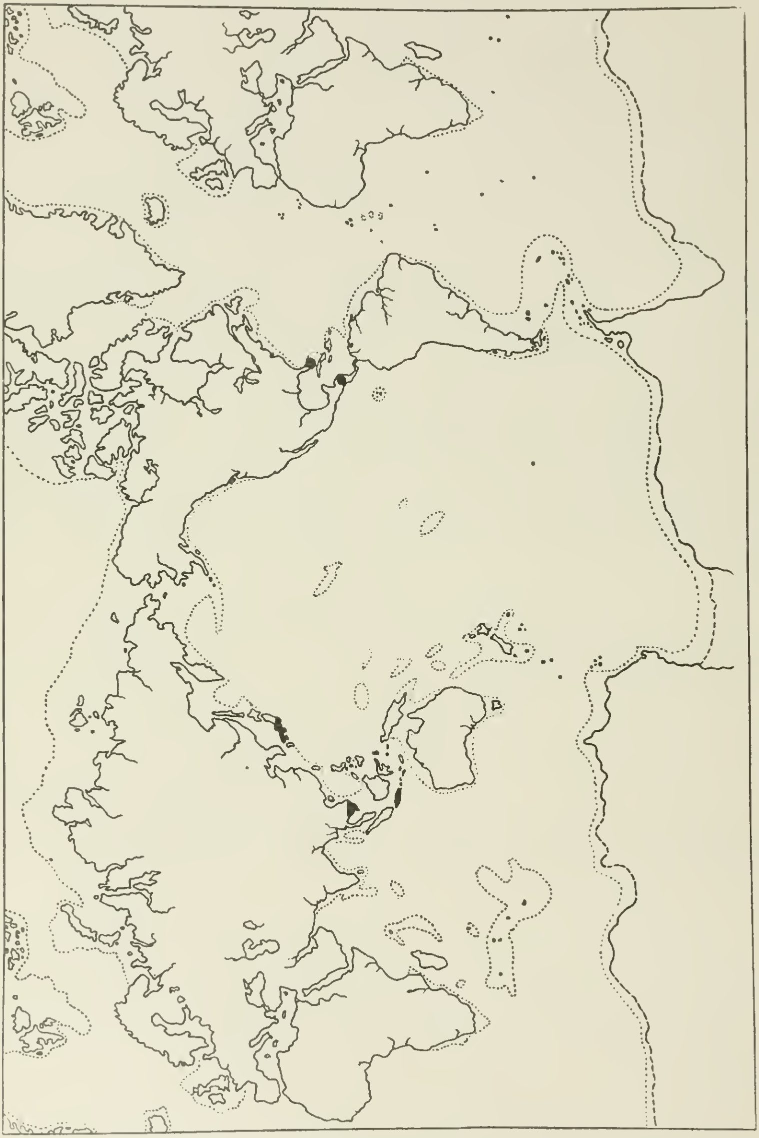

FIG. 252.—THE DISTRIBUTION OF DIVISION 5 OF THE GENUS CEPEDEA.

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[33] *Scaphiopus albus* might well be treated as a subspecies of *S. solitarius* (Holbrook).

Scaphiopus known to bear *Cepedea*, *Protoopalina* being the usual Opalinid in this American genus, though one *Zelleriella* and two *Opalinae* have been reported. We have no record of *Scaphiopus* in South America or in Central America south of northern Mexico, and northern Mexico zoögeographically belongs to North America and not to tropical America. *Scaphiopus albus*, or its ancestors, probably, therefore, reached Key West from southwestern United States by a route north of the Gulf of Mexico rather than by way of tropical Central America and either of the land bridges from this region to the West Indian lands and on to Florida. Probably, then, its *Cepedea* is derived from some species in western North America. If we are correct in regarding *C. occidentalis* and *C. floridensis* as related to the southeastern Asian *C. pulchra*, then we must believe that their forbears entered America from Asia by way of Alaska and spread across temperate America to the tip of Florida, a migration during the evolution of these species of *Cepedea* which almost rivals that of the Hylas from tropical South America (their ancestral home) north to Alaska and west to westernmost Europe. The present distribution of the species of division 5 and comparison of the charts would indicate origin in the Indian island in the later Cretaceous (figs. 235, 235, *A*), or in the early Tertiary (fig. 236), or in continental Asia or Malaysia in the late Tertiary (fig. 237) or later (fig. 238), and spreading to their present homes—from Asia to Malaysia or *vice versa*, to Japan, and by way of Alaska to North America and Central America.

Division 6 (fig. 253).

C. hispanica (p. 161)	in Rana	Spain.
C. cantabrigensis (p. 162)	in Rana	Northwestern North America.
C. multiformis (p. 164)	in Hyla	tropical Central America, Brazil.
C. multiformis [of Polypedates schlegelii] (p. 165)	in P. schlegelii (Raninae),	Japan.
C. seychellensis (p. 167)	in Magalixalus (Raninae)	Seychelles Islands.
C. doliochosoma (p. 168)	in Bufo	Tropical Central America
C. sp.? (p. 170)	in Hyla	Texas.
C. longa (p. 168)	in Rana	"Asia," Japan, Formosa.
C. ophis (p. 170)	in Rana	Formosa, East Indies.
C. segmentata (p. 171)	in Polyedates (Raninae)	Cochin China, East Indies.

In this division we place a group of species which, on the one hand, approach the *dimidiata* group and, on the other hand, have a greatly elongated body form, and, between, show a series of intergrading species. *Cepedea hispanica*, which most nearly approaches the *dimidiata* group, is a southwestern European form, living in a region now inhabited by two species of the *dimidiata* group. It may not be closely related to the other species placed in this group, but seems to be so. The most highly evolved members of division 6, *C. ophis*, *C. longa*, and *C. segmentata*, are found in Malaysia and eastern Asia. It is in these species that the evolution of the genus culminates, and in respect to the strange inhibition of division, characteristic of the Opalinidae, we may say that the evolution of the family culminates in *C. segmentata*. The other species intergrade in about the order named.

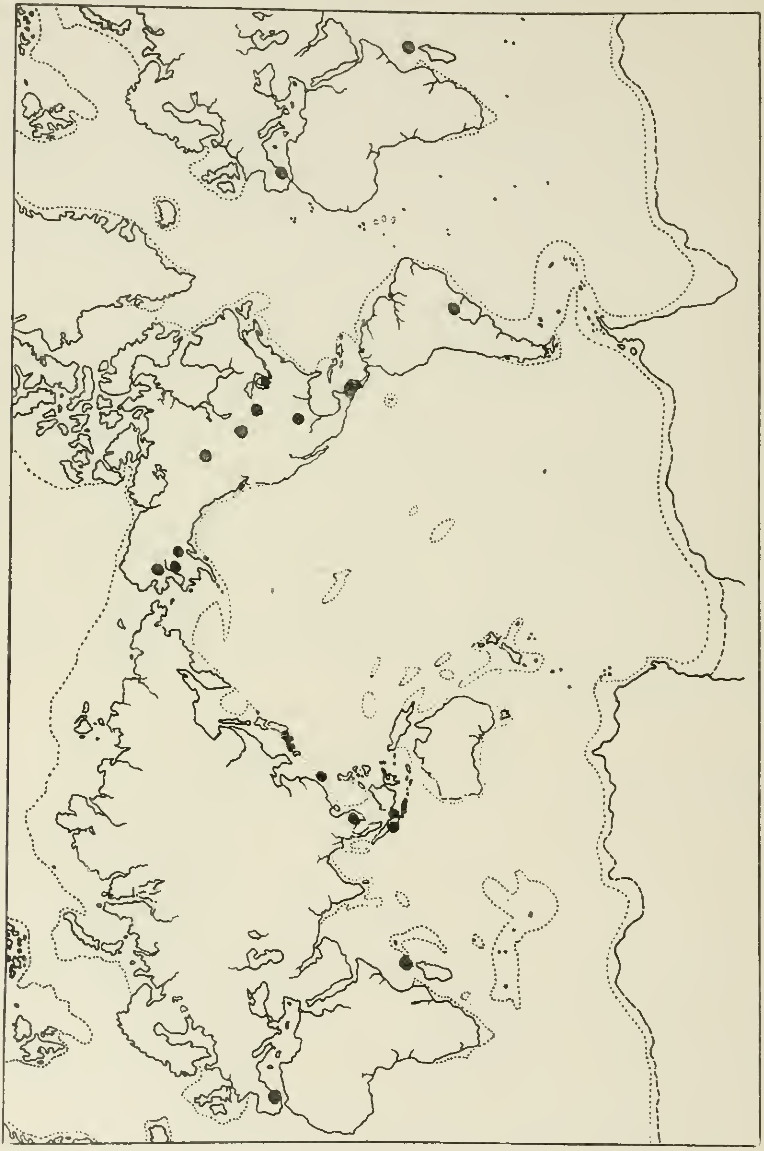

Fig. 253.—The distribution of division 6 of the genus Cepedea.

Representatives of this division are now found in Europe, Asia, and Malaysia, the Seychelles Islands off Madagascar, tropical Central America, and southwestern, northern, and northwestern North America. It is unfortunate that Indian Anura have been so little explored for Opalinids, for thorough knowledge of southern Asian Opalinids might give light upon the presence of a member of this group of Cepedeas in the Seychelles. Its presence in the Seychelles and the absence of related Cepedeas in tropical Africa suggest that it reached its present habitat by the Ceylon-Madagascar bridge at a comparatively late period when the Seychelles-Madagascar-Africa connection had been interrupted. But there is zoögeographic evidence from other sources that the Madagascar-Africa connection persisted longer than the Madagascar-Ceylon connection (fig. 236). We have here a bit of evidence that Madagascar separated from the trans-Indian Ocean bridge before the Seychelles did so. The data, however, are very scant and are not worthy of much emphasis. At best, considering the nature and small amount of our material from some regions, negative evidence, that is, absence of Opalinids of any sorts from one of these areas, is of very little weight. But when we have had a more comprehensive survey of these regional faunas the negative data may be emphasized.

The absence of Cepedeas of this group from Africa and their presence in eastern Asia and America seems to indicate migration to America from eastern Asia by way of Alaska. This is further borne out by the presence of *C. cantabrigensis* in extreme northwestern North America. Representatives of the group have passed south into Texas, tropical Central America, and Brazil. Here again we have evidence of extensive migration. From their present host distribution it seems these Cepedeas came to America in some *Rana* and that some of them were adopted by an American *Bufo* and two American Hylas.

The table for division 6, above, shows one noteworthy fact, namely, that almost identical Cepedeas (*multiformis*) occur in a Central American *Hyla* and a Japanese *Rana*. Forms like *C. multiformis* and the similar *C. cantabrigensis* may well have been the original immigrants from Asia. If so, the presence of these forms, so little modified to-day, emphasizes a fact of great interest, of which there is much evidence from many sources, namely, that the secluded and uniform habitat in which many internal parasites live allows their persistence for a long time without modification. This may be the explanation of the eastern hemisphere and western hemisphere forms being sometimes so similar that one feels compelled to place them in the same species, though recognizing subspecific differences. Another similar instance is the close resemblance between the Australian and South American Zelleriellas. Such modification as the Opalinidae have undergone since the establishment of their fundamental character has been due probably in large measure to trends within the Opalinids themselves rather than to environmental stimuli and control. Orthogenesis, rather than natural selection, seems to have been the chief factor in the evolution of the Opalinids.

Summarizing the indications as to division 6 of the Cepedeas we may say that apparently they evolved in India in the middle Cretaceous; that they spread to the Seychelles at about the time the India-Ceylon-Madagascar-Africa bridge was breaking up (fig. 235), for none of their members reached Madagascar or Africa; that they lived through the early Tertiary in the Indian island (fig. 236), reaching Asia in the late Tertiary (fig. 237) when India became joined to continental Asia; that during the late Tertiary or later (fig. 238) they migrated to Formosa and Japan (and possibly to Europe, *C. hispanica*), and that at the same time they spread to America by way of Alaska. The Brazilian species probably wandered southeastward from westernmost North America instead of crossing early from Africa, for no Cepedeas of this division are known from Africa.

UNGROUPED SPECIES.

The remaining species of *Cepedea* are difficult to associate in groups, for their affinities are not well indicated. Their geographical distribution is, therefore, of less interest to discuss. *Cepedea borneonensis* (p. 159), of Borneo, may well be a recently evolved species; *C. formosae* (p. 160), known from Hong Kong and Formosa, could have passed between its present habitats at any time since the middle of the Tertiary (fig. 237, p. 305); *C. fujiensis* (p. 159) may have evolved in Japan very recently, since Japan separated from the continent of Asia; the central American species, *C. mexicana* (p. 160), is of uncertain ancestry and does not disclose the geographical origin or migration of itself or its ancestors.

Summarizing the indications as to the genus *Cepedea* we may say that it apparently arose in India or in the Madagascar-India ridge during the Jurassic or early Cretaceous, before Africa and South America separated, but after Australasia had broken away from Equatoria; that it reached continental Asia from India in the later Tertiary and then spread to Europe, eastern Asia, Japan, and to the Western Hemisphere by way of Alaska. One of its South American representatives apparently arose from immigrants from Africa.

The hosts and the geographical occurrence of the species of the genus *Opalina* are given in the following table, in which the *Opalinae angustae* are annotated [*A*] and the *Opalinae latae* are annotated

[*L*]. while a few species of doubtful classification are annotated [*L ?*].

Salamandridae (1 species of host, 1 species of *Opalina*).
- *Triturus alpestris* (*O. ranarum* [L]); Switzerland.

Dissoglossidae (1 species of host, 1 species of *Opalina*).
- *Bombina bombina* (*O. ranarum* [*L.*]); Switzerland.

Pelobatidae (2 species of hosts, 3 species of *Opalina*).
- *Scaphiopus hammondii* (*O. oblanceolata* [*A*]); northern Mexico, Arizona.
- *Scaphiopus solitarius* (*O. obtrigonoidea*, [*A*]); North Carolina.

Hylidae (24 species and subspecies of hosts, 16 or 17 species and subspecies of *Opalina*).
- *Acris gryllus* (*O. virguloidea magninucleata*, [*A*]);North Carolina.
- *Agalychnis helenae* (*O. helenae*, [*A*]); Nicaragua, Costa Rica.
- *Agalychnis moreletei* (*O. moreletei* [*L?*]); Guatemala.
- *Chorophilus feriarum* (*O. chrophili*, [*A*]); North Carolina, District of Columbia.
- *Chorophilus occidentalis* (*O. chorophili*, [*A*]); Georgia, Florida.
- *Chorophilus ornatus* (*O. chrophili*, [*A*]); Texas.
- *Chorophilus triscriatus* (*O. chrophili*, [*A*]); North Carolina, northern Ohio.
- *Hyla arborea* (*O. obtrigona*, [*A*]); central Europe.
- *Hyla arborea japonica* (*O. obtrigona*, [*A*]); Japan, Manchuria.
- *Hyla arborea savignyi* (*O. obtrigona*, [*A*]); Jerusalem.
- *Hyla arenicolor* (*O. obtrigonoidea*, [*A*]); Arizona.
- *Hyla baudinii* (*O. guatemalae*, [*L?*]); Guatemala, Mexico.
- *Hyla cinerea* (*O. obtrigonoidea orbiculata*, [*A*]); Texas, Louisiana, Florida, Maryland.
- *Hyla evittata* (*O. terrae-maria*, [*L?*]; Maryland.
- *Hyla eximia* (*O. virguloidea*, [*A*]); Mexico.
- *Hyla femoralis* (*O. obtrigonoidea*, [*A*]); Georgia.
- *Hyla pickeringii* (*O. obtrigonoidea*, [*A*]); Illinois.
- *Hyla pickeringii* (*O. pickeringii*, [*A*]); North Carolina. District of Columbia, Ohio, British America.
- *Hyla regilla* (*O. oregonensis*, [*A*]); Vancouver Island.
- *Hyla regilla* (*O. virguloidea*, [*A*]); Los Angeles County. California.
- *Hyla septentrionalis* (*Opalina* sp. [*A*]), Cuba.
- *Hyla versicolor* (*O. hylaxena*, [*A*]); Michigan, Massachusetts, Georgia.
- *Hyla versicolor* (*O. hylaxena parvinucleata*, [*A*]); Michigan, Massachusetts, Georgia.
- *Hyla versicolor chrysoscelis* (*O.* sp. ?, [*A*]); Texas.

Phyllomedusa dacnicolor (*O. helenae phyllomedusae*, [*A*]); Mexico.

Bufonidae (16 species and subspecies of hosts, 16 species and subspecies of *Opalina*).

Bufo boreas (*O. obtrigonoidea maxima*, [*A*]); western Canada. Alaska.
Bufo bufo (*O. cincta*, [*L*]); France.
Bufo bufo (*O. ranarum*, [*L*]); Europe.
Bufo bufo (*O. bufoxena*, [*L*]); southern Manchuria.
Bufo bufo asiaticus (*O. asiaticus*, [*L*]); Shanghai, China.
Bufo cognatus (*O. discophrya*, [*A*]); New Mexico.
Bufo compactilis (*O. gigantea*, [*L*]); Texas.
Bufo compactilis (*O. spiralis*, [*A*]); Texas, Arizona.
Bufo copei (*O. discophrya*, [*A*]); northern and north-eastern Canada.
Bufo fowleri (*O. obtrigonoidea*, [*A*]); North Carolina, Massachusetts.
Bufo haematiticus (*O.* sp. ?, [*L?*]); Nicaragua.
Bufo halophilus (*O. obtrigonoidea maxima*, [*A*]); San Francisco, California.
Bufo lentiginosus (*O. triangulata*, [*L*?]); Florida.
Bufo punctatus (*O. obtrigonoidea*, [*A*]); Guanajuato, Mexico.
Bufo raddei (*O. raddei*, [*L*]); China.
Bufo smithi (*O. ranarum smithi*, [*L*]); Japan.
Bufo typhonius (*O. panamensis*, [*L*]); Panama.
Bufo viridis (*O. ranarum*, [*L*]); southern Europe.
Bufo woodhousi (*O. woodhousi*, [*A*]); Arizona, Utah.

Gastrophrynidae (1 species of host, 1 species of *Opalina*).

Gastrophryne carolinensis (*O. obtrigonoidea*, [*A*]); Georgia, Virginia.

Ranidae (25 species and subspecies of hosts, 23 species and subspecies of *Opalina*.

Hylambates rufus (*O. camerunensis*, [*L*]); the Cameroons.
Phrynobatrachus natalensis (*O. natalensis*, [*L*]); Sudan.
Polypedates maculatus (*O. virgula*, [*A*]); Ceylon.
Rana aesopus (*O. obtrigonoidea lata*, [*A*]); Florida.
Rana areolata (*O. kennicotti*, [*A*]); northern Illinois.
Rana arvalis (*O. ranarum* form *arvalis*, [*L*]); lower Austria.
Rana aurora (*O. obtrigonoidea lata*, [*A*]); Oregon.
Rana boylei (*O. virguloidea*, [*A*]); San Francisco, California.
Rana clamitans ? (*O.* [*larvarum*], [*A*]); Nova Scotia.
Rana copei (*O. copei*, [*L* ?]); Costa Rica.
Rana cyanophlyctis (*O. coracoidea*, [*L*]); "Asia."
Rana dalmatina (*O. ranarum*, [*L*]); Bosnia.

Rana draytonii (*O. draytonii*, [*L*]); Pacific coast of the United States.
Rana erythraea (*O. rotunda*, [*L*]): Siamese Cambodia.
Rana esculenta (*O. ranarum*, [*L*]); Switzerland.
Rana japonica (*O. japonica*, [*L*]); Japan.
Rana limnocharis (*O. japonica*, ?, [*L*]); Java.
Rana limnocharis (*O. lata*, [*L*]); "Asia."
Rana mascareniensis (*O.* sp. ?, [*L*]); Gold Coast. Africa.
Rana palustris (*O. obtrigonoidea*, [*A*]); Ohio, Massachusetts.
Rana pipiens (*O. obtrigonoidea*. [*A*]); North Carolina. Ohio, Illinois.
Rana pipiens austricola (*O. obtrigonoidea austricola*. [*A*]): Costa Rica; Guatemala; Tabasco, Mexico.
Rana pipiens sphenocephala (*O. carolinensis*. [*A*]); Florida, South Carolina.
Rana pretiosa (*O. copei*, [*L* ?]); Montana.
Rana septentrionalis (*Op.* sp. ? [*A*]); Ontario.
Rana sylvatica (*O. virguloidea*, [*A*]); Ohio, North Carolina.
Rana temporaria (*O. ranarum*, [*L*]); Europe, Japan.
Rana temporaria (*O. ranarum* form *cinctoidea*, [*L*]); Germany.
Rana temporaria (*O. ranarum* form *truncata*, [*L*]); Germany.
Rana temporaria parvipalmata (*O. ranarum parvipalmatae*, [*L*]); France.

The species of the genus *Opalina* show two divergent groups' *Opalinae angustae*, forms which are long and narrow. especially behind, like *O. obtrigona;* and *Opalinae latae*, which are broad like *O. ranarum* or *O. cincta.* The *Opalinae angustae* may be vaguely divided into the curved, *virgula*-like species and the straighter, *obtrigona*-like species, but this distinction is vague and of little interest. The *Opalinae angustae*, with two exceptions, are Western Hemisphere forms, the *Ophalinae latae*, with possibly from three to five exceptions, are found in the Eastern Hemisphere. One Discoglossid is reported to harbor an *Opalina* (*ranarum*). a very rare occurrence. of which there is but a single report. It may not have been a permanent infection. In the Pelobatidae the only Opalinas known (3 species) are of the narrow group. In the Hylidae the *obtrigona*-like froms predominate. though there are five Opalinas of the virguloid form. All these, of course, are narrow, though in three of the *obtrigona*-like species some broad individuals. almost *ranarum-like*, are seen along with the slenderer individuals. The Bufonidae show about equal numbers of the narrow and the broad Opalinids. From one species of the Gastrophrynidae. the only one known to be infected with *Opalina*, we have a single species of *obtrigona*-like *Opalina.* No *Opalinae* are known from the *Dendrobatinae.* They are very numerous among the *Raninae;* a few are of the *obtrigona* group, and still fewer are *virgula*-like; most are of the broad form. We thus see that the narrow species are most abundant in the less modified families of the Anura, and that the broad species are numerous in the Bufonidae, and are dominant in the *Raninae*. Several Hylids bear species which are somewhat intermediate in form between the narrow and broad species. These are all of course secondarily adopted guests, for *Opalina* was not originally present in tropical South America, the early home of the Hylidae. (See the next page.)

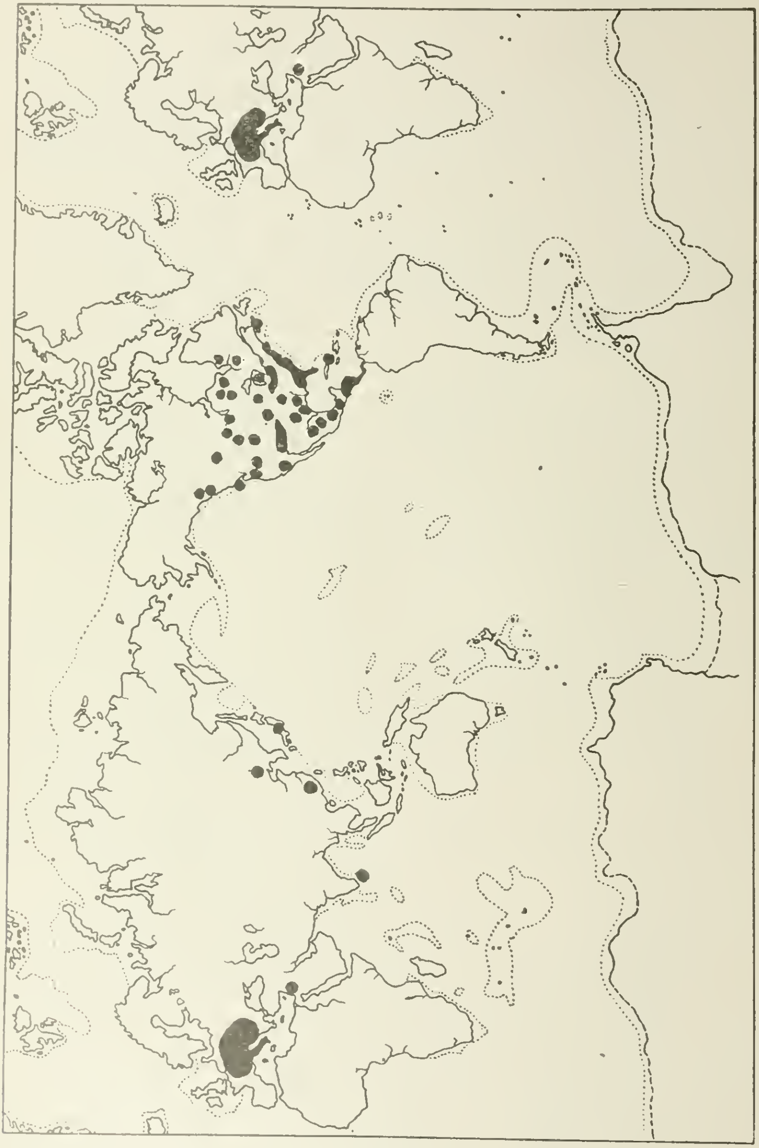

FIG. 254.—THE DISTRIBUTION OF THE OPALINAE ANGUSTAE. THE CEYLONESE FORM IS DOUBTFUL. ALL THE PALEARCTIC RECORDS ARE OF THE ONE SPECIES OPALINA OBTRIGONA.

But the data should also be scrutinized from the point of view of geographical distribution (fig. 254.) The group of *obtrigona*-like species (straight or slightly bent, narrow forms) is represented, *a*, in 3 species of *Scaphiopus*, a North American Pelobatid; *b*, in 4 species of *Chorophilus*, an American Hylid; *c*, in 16 species of the genus *Hyla*, 9 of them from North America, 1 from Central America, 1 from Cuba, and 1 from Europe with its 2 subspecies extending into all temperate Asia; *d*, in 2 Central American species of *Agalychnis*, a genus of the family Hylidae; *e*, in one *Phyllomedusa*, a Mexican Hylid; *f*, in 9 Bufos all from Central America or North America; *g*, in 1 Gastrophrynid from southeastern United States; *h*, in [34] species of *Rana* from Central America or North America; there are also 5 other species from Central America and North America which are intermediate forms, 3 intermediate between the *obtrigona* group and the *virgula* group, and 2 intermediate between the *obtrigona* group and the *ranarum* group. We thus see that the *obtrigona*-like species are exclusively Central American and North American, except for the one species *O. obtrigona* which has passed from North America into Asia and across into Europe, being carried by *Hyla arborea*, the only species of *Hyla* found in Asia and Europe, except for two "closely related" forms in eastern Asia, and an Abyssinian species of doubtful relationship.

The *virgula*-like species are not very different from the *obtrigona*-like forms, and there are four intermediate species. The only marked difference is the strongly curved body. *Virgula*-like species are known, *a* from three Hylas, all from western North America; *b*, from two Ranas, one from the eastern part, the other from the western part of the United States; *c*, from *Polypedates* (?) *maculatus* from Ceylon—a most surprising occurrence which needs careful scrutiny. Excepting *Opalina virgula* itself, the Ceylonese form, the *virgula*-like species are so similar to *obtrigona*-like species in structure and distribution that they may be treated together as one group. *Opalina virgula*, on the other hand, is demarcated by its long, slender, rod-like, endoplasmic plastids. I am inclined to believe it should be regarded as distinct in origin from the other *Opalinae angustae*, and that it probably arose independently from some *Cepedea*. We should have fuller knowledge of Indian material. Removing *Opalina virgula*, then, from the other narrow *Opalinae*, we may say that all the latter are Western Hemisphere species, except that *O. obtrigona* has secondarily invaded northern Asia and Europe by way of Alaska. Note that though we find in Australia Anura belonging to the same families whose members in America harbor slender Opalinas. the Australian members of these families do not, so far as the records go, have these *obtrigona*-like or *virgula*-like Ophalinas. Indeed no member of the genus *Opalina*, whether slender or broad, straight or curved, is known from Australasia, and no *Cepedea* either. That is, no multinucleated Opalinids are Australasian.

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[34] *O.* [*lawarum*] seems to be a narrow *Opalina*, but its adult condition has not been seen.

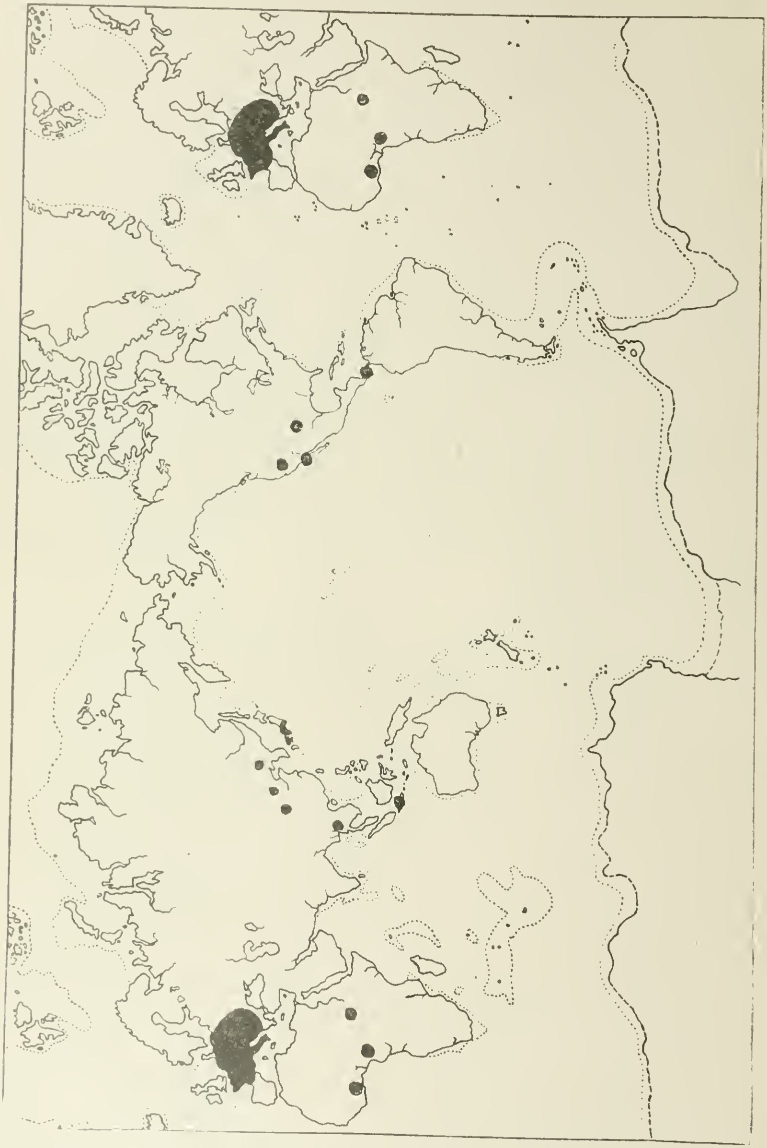

FIG. 255.—THE DISTRIBUTION OF THE OPALINAE LATAE.

It is noteworthy that no species of the genus *Opalina* is known from South America, although both narrow and broad species are found in tropical Central America. The *Opalinae angustae* are all

American in their origin (except *O. virgula* in a *Polypedates* (?). from Ceylon) and are a northern group and *Opalina virgula* may have been derived independently from *Cepedea*, as noted above. *Opalina obtrigona* has migrated to Asia and Europe (in *Hyla arborea*), but is still confined to the North Temperate Zone. Of the broad Opalinas in the Eastern Hemisphere (fig. 255) all are north temperate forms except one species from Siamese Cambodia (*O. rotunda* in *Rana erythroea*), one species from Java (*O. japonica* (?) in *Rana limnocharis*), and three species from tropical Africa (*O. camerunensis* in *Hylambates*, a Randid, *O. natalensis* in *Phrynobatrachus*, a Sudanese Ranid, and an unnamed species from *Rana mascareniensis*). Of the species and subspecies of *Opalina*, whose locality is known, 28 narrow forms and 10 broad forms are northern and 5 wide forms are southern. This statement omits 6 forms intermediate in shape, all northern, and it includes Central American and West Indian forms as northern, for they evidently came from the north. *Opalina virgula* also is omitted from consideration. The distribution of the *Opalinae*, both narrow and broad, seems to indicate they are northern in origin as well as in present habitat.

How did the African species of *Opalinae latae* reach their present home? They did not come from South America, for no Opalinas of any sort are known from South America. They must have reached Africa from Euro-Asia in the late Tertiary (fig. 237, p. 305) or in the Pleistocene (fig. 238), a migration corresponding in time and route to that of *Bufo* and *Rana*, in one or both of which hosts the *Opalinae* probably entered tropical Africa.

The presence of a narrow *Opalina* (sp. ?) in a Cuban *Hyla* (*septentrionalis*) is of much interest. It indicates the persistence of the Antilles-Central American bridge until after broad *Opalinas* had reached America from *Asia*, had there met *Hyla* and in *Hyla* had been changed into the narrow form. On geological evidence Vaughan (1919) puts the two Central America-Antilles bridges in the Pliocene. This would agree with the distribution data.

The Ranidae and Bufonidae are families with an abundant development in the northern regions of both hemispheres. Their characteristic parasites are *Protoopalina*, *Cepedea* and both broad and narrow forms of *Opalina*. The eastern frogs and toads have the broad *Opalinae*, the western frogs and toads have chiefly the narrow *Opalinae*, but show some species which grade toward the broad forms. Especially anterior daughter cells, after fission, are broad and may show no trace of a pointed posterior end. These might readily be mistaken for individuals of the *ranarum*-like group. On the other hand, some of the forms of *O. ranarum* itself, and of other species of its group, are somewhat narrow posteriorly, but the actual posterior end is usually not sharp pointed or even narrow pointed, but is well rounded or even indented. In the table on page 347 the slender species, some of whose individuals approach the *ranarum* group in shape, are indicated by the annotation "[*L* ?]" Of these *O. guatemalae* belongs apparently to the *Opalinae angustae*, so do *O. obtrigonoidea orbiculata*, *O. terrae-mariae*, *O. moreletei*, *O.* sp. (?) from *Bufo haematiticus*, and *O. copei.* On the other hand, *O. gigantea* and *O. draytonii*, though western species, are broad. *Opalina panamensis* may also be an immigrant broad species. We may say that the western species of *Opalina* are in general slender, or at least pointed behind, though some species show individuals which are broader throughout or not pointed behind.

On the other hand, omitting from consideration the problematic *Opalina virgula*, all but one of the known Eastern Hemisphere species of *Opalinae* are *Opalinae latae.* They occur, 1, in *Bufo* from Europe across to eastern Asia in the species *B. bufo* and its subspecies *asiaticus*, in *B. viridis* and in *B. smithi;* 2, in Ranidae, including *a*, Ranas from Europe, temperate Asia, southeastern Asia, and Java, also one *Rana* from the African Gold Coast; *b*, a *Hylambates* from the Cameroons; and *c*, a *Phrynobatrachus* from the Sudan. The *Opalinae latae* are thus seen to occur in Europe, Asia, and Africa. They are not known from Madagascar, Australasia, or South America. Two, or probably three, broad forms (*O. draytonii*, *O. gigantea*, and probably *O. panamensis*) occur in North America and Central America. It seems that these are immigrants from Asia by way of Bering Straits. Other American species which show some broad individuals show also other narrower individuals which indicate affinity with the *Opalinae angustae.*

The line of demarcation between the *Opalinae anguatae* and the *Opalinae latae* is not a sharply indicated one, but it is clear that the eastern species are broad and the western species are in general narrower. It is also clear that the *Opalinae* are of northern origin and have not extended to any degree to the southern hemisphere except as recent immigrants into Africa.

Opalina doubtless evolved from *Cepedea* and so, of course, is more recent. The Ranidae and the species of the genus *Bufo* are to-day the chief hosts of the eastern Opalinas (*Opalinae latae*) and in either the Ranidae or, less probably, *Bufo* the broad Opalinas may well have evolved. *Rana*, *Bufo*, and especially the North American and Central American Hylidae are to-day the chief hosts of the western Opalinas (*Opalinae angustae*). Apparently in one of these three groups, probably in the Hylidae, these narrow western forms first arose. Their evolution in some Hylid, rather than in *Bufo* or a Ranid, is probable, for they are not known in Bufos or Ranids except such as in America have come into extensive contact with the Hylidae. The European *Hyla* which bears a narrow *Opalina* has not succeeded in infecting any European *Rana* or *Bufo*. It was very likely the change from the former host to a Hylid which stimulated the adopted broad *Opalina* to diverge to the narrow form. The narrow *Opalinae* probably evolved quite late, for they have not yet spread to South America, although any southward spread of the Ranas, Bufos, or Hylids might well carry the narrow *Opalinae* also. There has probably been land connection between North America and South America by way of Central America since the latest Miocene or more probably the Pliocene (fig. 237, p. 305). If the *Opalinae angustae* first evolved in a Hylid from a broad *Opalina* adopted in Central America or North America, this probably occurred quite late, for two reasons: First, it must have been after the broad Opalinas had evolved in Euro-Asia and had migrated to North America, and the genus *Opalina* is apparently of later Tertiary origin, having evolved from Cepedea after this genus had reached Asia proper from India (figs. 236, 237; also see p. 346); second, it must have been after the Hylidae reached Central America coming from tropical South America, and the Brazilian home of the Hylids could hardly have been united to the Ecuador-Central America-California-Alaska-Siberia land strip until after it had become interrupted, probably in the north, else the Hylids would have passed on in numbers into Asia. This places the evolution of the *Opalinae angustae* from the *Opalinae latae* in the Miocene or Pliocene. If it occurred in the Pliocene, the Hylids may not have reached Central America until the Isthmus of Panama was established. If the evolution of the narrow Opalinas occurred as early as the Miocene, the Hylids must then have already passed to Central America by way of the eastern Pacific land strip. The later date is the more probable, the Isthmus being probably of Pliocene origin.

Summarizing the evidence as to the *Opalinae*, some of which will soon be developed more fully in discussion connected with the several families of Anura, we may say: First, as to the *Opalinae latae*, that their absence from South America indicates that they were not in South Atlantis in Jurassic times or early Cretaceous times (fig. 233); that they apparently evolved from *Cepedea* in southeastern Asia or the Malay Islands after *Cepedea* had reached continental Asia in the late Tertiary (fig. 237), as discussed on page 346; that during this period, or during the Pleistocene (fig. 238), they entered Japan, Europe, Africa, and America by way of Siberia; that they probably entered America before the earliest glacial period, for a narrow *Opalina* had time to evolve from them in America and spread back across to Siberia and all Euro-Asia. Of the *Opalinae angustae* we may say: That they evolved in North America, or possibly in Central

America, from some broad *Opalina*, and probably in some species of the Hylidae which adopted and proceeded to modify this broad *Opalina;* that this could not have occurred earlier than the Miocene or more probably the Pliocene, for Hylids were not in Central or North America before the Miocene or Pliocene; that this transformation of broad Opalinas into narrow Opalinas occurred before the earliest glacial period, since a narrow *Opalina* was carried by a *Hyla* to Euro-Asia by way of Alaska, a migration improbable after the first glaciation of the Quaternary; that the *Opalinae angustae* are the most recently evolved of the groups of Opalinidae, they, and probably also their ancestors the *Opalinae latae*, being even more modern than the Zelleriellas.

One of the most interesting results of our study of the Opalinidae is seen in the indication that ancient subgeneric groups, some of them probably of Triassic origin, are still extant. For example, a group of species of the genus *Protoopalina* (including *P. acuta* and *P. papuensis* from Australia, *P. diplocarya* from South America, and *P. xenopodos* from tropical Africa), archaic in character, are found to-day so distributed in the three great southern continents as to be an indication of their origin in the Triassic period, when these three land areas were united to form the continent Equatoria. In this and in other instances, especially in the genus *Zelleriella*, we find very closely similar species present in widely separated areas—group 1 of the genus *Protoopalina* in Africa, South America, Australia, and Papua; *Protoopalina*, group 4, in Europe and Australia; *Protoopalina*, group 8, in Africa and South America; almost identical species of *Zelleriella* in South America and Australia; *Cepedea*, division 2, in Euro-Asia and South America; *Cepedea*, division 4, in Africa and Florida; *Cepedea*, division 5, in Asia and tropical America; *Cepedea*, division 6, in the Seychelles, Malaysia, Asia, and in North America; and *Opalinae angustae* in North America and Euro-Asia.

It would be difficult to parallel these conditions from many groups of free-living animals. Apparently it is chiefly the parasitic life, in which the animals are protected and fed and are without much variety of environing conditions to make divergent adaptation advantageous, that has allowed the Opalinids to persist through a number of geologic periods with so little modification in numerous instances. Of course there was an early and very considerable modification to fit these organisms in the first place to their habitat within their hosts. Their loss of a "mouth" was the most noticeable structural feature in this early modification. There has been a good deal of divergence since the parasitic habit was acquired, so that now we may recognize two subfamilies, four genera, and certain subgeneric groups of species. But among the Opalinids we find to-day numerous species which have persisted for several geologic periods substantially unmodified.

This persistence of archaic forms is a rather widely spread phenomenon among parasites. Having once met the conditions of parasitism and having undergone the initial modification to adapt them to the conditions of the new environment, some of their species are rather prone to persist without much further change, living as they do in a secluded, protected, and remarkably uniform habitat. Parasites may thus present peculiarly dependable evidence in such questions as we have discussed in this chapter.

How long a period has been required for the evolution of the Opalinidae and their four genera? We have seen indication that *Protoopalina* was present in the Triassic period, and it may have evolved earlier. There are present today in Australia, Papua, tropical Africa, and South America, species which are very closely similar to one another and which probably are in almost the same condition which they had reached during the Triassic. Other groups of species of the Archais genus *Protoopalina* seem to have arisen in the Jurassic or late Cretaceous (groups 3, 4, 6 ?, 7 ?, and 9), in the middle or later Cretaceous (groups 5, 6 ?, and 7 ?), and in the Tertiary (group 8). The genus *Zelleriella* evolved from *Protoopalina* apparently during the Miocene. *Cepedea* evolved from Protoopalina probably during the Jurassic or late Cretaceous. The period of origin of some of its groups of species does not seem to be indicated by our data (divisions 2 and 3). The time of origin of other groups is indicated. Division 1, the most archaic division, must, of course, have arisen as early as the Jurassic or early Cretaceous, and division 2 probably arose during the same period. Division 6 apparently arose late in the Cretaceous. Division 5 seems to be of Tertiary origin, and the Japanese species, *C. fujiensis*, may well be of quite late origin, since the time when Japan became isolated from continental Asia. The earliest *Opalinae* were the *Opalinae latae*, which doubtless evolved from *Cepedea*. They apparently arose at some time after the middle of the Tertiary and not later than the Pliocene. The *Opalinae angustae* evolved from the *Opalinae latae*, probably during the Pliocene.

There is another aspect of the matter that is of interest. Secluded and protected in their uniform habitat, the Opalinidae have been but little exposed to the selective action of the struggle for existence. Under these conditions orthogenetic trends have had unusually free play and the resultant speciation is to an unusual degree an expression of the undisturbed outworking of the natural tendencies in the animals themselves.

Unfortunately for the study of orthogenetic trends, conditions of internal parasitism tend to cause an early simplification in structure, involving among higher forms degeneration in the alimentary system, the sense organs and the locomotor organs, including the muscles and the nervous mechanism connected with the locomotor system and the sense organs. There is, therefore, a narrower range of structural features within which the orthogenetic trends may express themselves. But Protozoa which live unattached within the host, as do most of the endoparasitic Ciliates, lead about as active lives as they would in a pond. They are protected from enemies without becoming greatly degenerate. Many retain their original alimentary apparatus and habit, being not parasites so much as scavengers. The Opalinidae are hardly true parasites, for though they live upon predigested food, to be sure, it is only upon such of this food as is present in the fecal mass and was destined probably to be thrown away. But the Opalinids have gone further than *Nyctotherus* and *Balantidium*, for example, in their adaptation to parasitism, for they use predigested food and, in consequence, have lost their ingestion apparatus and have doubtless modified their physiological processes. But once these initial modifications were secured, their further evolution and speciation has apparently been due chiefly to orthogenetic trends free to an unusual degree from hindrance or guidance from any selective conditions in their environment. The present condition, therefore, of the family is in unusal measure an expression of the real nature of the organisms themselves. Environmental opportunities have been limited and environmental pressure has been less strong than in the case of free-living organisms.

THE ORIGIN AND THE SPREAD OF THE SEVERAL GROUPS OF THE ANURA.

The problems and evidence as to the dates and places of origin and the spreading of the Anura and their Opalinid parasites are so interwoven that it is difficult, in their discussion, to avoid passing constantly back and forth between the hosts and their parasites. Data from each side must be brought into the discussion of the problems of the other. We have tried, however, in the first portion of the discussion in this section to deal mainly with the parasites. In the next few pages we will deal mainly with the hosts, and in the final portion of this section we will treat some problems from the double viewpoint.

The Pipidae (fig. 222, p. 286).

The Pipidae are usually considered the most archaic of the extant families of the Anura. They are represented in northeastern South America by one species, *Pipa pipa*, the Surinam Toad. In Africa we find two genera, *Xenopus* and *Hymenochirus*, which may be placed in a distinct subfamily, *Xenopodinae*, or possibly might even be elevated to family rank. The chief argument against regarding *Pipa* and the African genera as closely related forms has been, as Gadow (1909) states, their occurrence in regions so far apart and separated by the Atlantic Ocean. But the resemblance between the genera is such that most herpetologists have felt compelled to regard them as related. The parasites of these genera would be of especial interest in view of the problem of distribution. No specimens of *Hymenochirus* have been available. Three preserved specimens of *Xenopus muelleri* proved barren, so also did 22 specimens of *Xenopus laevis*. Most of these were very stiff from preservation in too strong alcohol and were in no condition for successful study. Three fine specimens of *Pipa* from the United States National Museum were opened, but with negative results. Nine specimens of this species were sent me by the American Museum of Natural History, through the kindness of Miss Dickerson, and these also proved uninfected. Of 20 specimens of *Xenopus calcaratus*, also from the American Museum of Natural History, 6 were found to bear *Protoopalina xenopodos*, a species with a long, slender, posterior point, recalling *P. acuta* of Australia and *P. diplonucleata of Chile*. The peculiar breeding habits of *Pipa pipa* may account for its having no Ciliate parasites, for the huge eggs and the larvae are carried upon the back of the female, and, so far as known, the larvae do not have any free-living, browsing, vegetarian stage in their development, the stage during which other Anuran tadpoles ingest Opalinid cysts and become infected.

There are known among the Pipidae three living genera: *Pipa*, in northern South America; *Xenopus*, with three species in central and southern Africa; *Hymenochirus*, from the same regions as *Xenopus;* and, besides these living genera, we know *Palaeobatrachus*, from mid-Tertiary deposits in central Europe. In all likelihood the South American representative of the family, or its ancestor, entered as an immigrant from Africa by the trans-Atlantic route. The absence of known fossil or modern Pipidae from Australia, southern South America, Asia, and North America would argue in favor of an origin of the family in Africa (fig. 233), with migration to northern South America in the late Jurassic or early Cretaceous, and with migration of one member of the family, *Paleobatrachus*, from Africa to Europe in the Middle Tertiary (fig. 237). It was apparently in early ancestors of the Pipidae, in Equatoria, that the Opalinidae arose, as early as Triassic times (p. 356). The Pipidae seem to be a waning family, and it is possible that they may have had a wider distribution in earlier times

The Discoglossidae (fig. 223, p. 287).

The Discoglossids have a peculiar, but not inexplicable, distribution. *Ascaphus* is found in extreme western North America. The genera *Alytes* and *Discoglossus* are European. *Bombina* is European and eastern Asiatic. Stejneger (1905, *a* and *b*), who has studied the distribution of this family, " makes the region southeast of the Himalayas, in Asia, the original home of the family. From here they radiated to New Zealand in early Cretaceous times,[35] to western America (over the land bridge that existed between Asia and North America) in upper Cretaceous times, and to western Europe in early Tertiary[36] times. Curiously enough, although at the moment of publication of his theory no Discoglossid toad had ever been found in the region indicated as the center of radiation, a new species [*Bombina maxima*, Tong Chang Fu, Province of Yunan] was announced from there one month later by Boulenger.[37]

The single specimen examined of the very local and rare *Ascaphus truei* was barren. *Alytes* bears *Cepedea minor. Bombina* carries four species of *Protoopalina* of the archaic *intestinalis* group and there is a single report of an infection by *Opalina ranarum.* No light is cast upon the distribution of the Discoglossidae by our incomplete infection data and we know no fossil forms, but the present occurrence of its members shows the family to have arisen probably from forms in southeastern Asia during the Cretaceous (fig. 234), or later, after Australia had severed its connection with Asia. Their spread to Europe may have been either in the Cretaceous (fig. 234) or during the Tertiary (figs. 236, 237). Their migration to extreme northwestern United States occurred probably in the late Cretaceous or early Tertiary, as is indicated by the fact that they are known only from the Olympic Mountains west of Puget Sound and from the coastal Siskiyou Mountains. The American genus, *Ascaphus*, apparently hadn't retained sufficient vigor for further spreading when in the later Tertiary the Olympic Mountain region became connected with lands further east.[38] They seem to be a waning family.

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[35] *Liopelma*, the only New Zealand amphibian, was classed as a Discoglossid at the time Dr. Stejneger wrote this sentence. He wrote me recently that *Liopelma* has now been found to be a Leptodactylid.

[36] Arldt would date each of these migrations, except the one to America, one period earlier, Australia severing its connection with Asia before the Cretaceous (fig. 234), and Europe being separated from Asia in the early Tertiary by an ocean strait from the Mediterranean Sea and the Indian Ocean, on the south, through the Caspian Sea to the Arctic Ocean (fig. 236).

[37] Quoted from Dickerson (1906), p. 52.

[38] It hardly seems best to discuss here the question of late Cretaceous or nearly Tertiary land connection from the Olympic Mountains eastward. It is simpler to align our data with Arldt's charts as given.

The PELOBATIDAE (fig. 224, p. 288).

The Pelobatidae are known from southern Europe, southern and eastern Asia and the East Indies, and from the United States of America and Mexico. *Megalophrys*, from Java, carries *Protoopalina montana. Pelobates*, from Europe, bears *Protoopalina pelobatidis* and "*P. intestinalis.*" *Pelodytes* from Europe has not been adequately studied, only a single individual having been opened, and this with negative result. The only American genus, *Scaphiopus*, bears *Protoopalina*, *Zelleriella*, a very doubtful *Cepedea* (*flava*) and narrow *Opalinae.* The Opalinid parasites of this family throw little light upon the problem of its geographical distribution. The American *Scaphiopus* was probably evolved in America from immigrant ancestors. Its *Zelleriella* parasites must have been adopted in Pliocene times or later in America, after southern South America, the home of the Zelleriellas, had united with tropical America. Its narrow *Opalinae* also are American. Its Cepedea (*floridensis*) was probably adopted from immigrant hosts coming from Siberia, or from their descendants. Its Protoöpalinas are characteristic forms, probably of American origin in this American genus of hosts. They resemble the species of the *intestinalis* group more than any others and probably were evolved from them, and this is added indication that the ancestors of *Scaphiopus* came to America from the Orient by way of Alaska, for Euro-Asia is the chief locality for the *intestinalis* group of Protoöpalinas.

This is an old family. Their absence from Australia, Africa and South America indicate a post-Triassic origin (fig. 232). Their occurrence in Papua is a puzzle. Bufonids, Pelobatids, and the ancestors of the Hylids and Leptodactylids probably all arose from related stocks and the place of origin was probably eastern Equatoria. The early Bufonids spread chiefly westward to Africa and South America, but are in Australia also. The Hylids and Leptodactylids arose in South America probably from the earliest Bufonids.

The ancestral branch which gave rise to the Pelobatidae moved northward from eastern Equatoria into the continent of Asia, probably just about the time Australasia separated from Asia-Malaysia, that is, during the last of the Jurassic (figs. 233, *A*, 234). They may have spread to Europe in Cretaceous times (fig. 234), or more likely during the later Tertiary (fig. 237), at which time Malaysia, which had been submerged during the Cretaceous and the early Tertiary, reemerged and received Pelobatids from southeastern Asia. The presence of Pelobatids in Papua and not in Australia is an indication, in line with evidence from Rana and the Gastrophrynids which we shall see later, that Papua had some connection with Malaysia after Papua and Australia had finally parted. Papuan paleogeography seems to need restudy. The Pelobatidae are apparently a decadent family and seem to be holding on chiefly through their adoption of a semi-subterranean habit.

The Bufonidae (fig. 225, p. 289).

The Bufonidae are cosmopolitan, within their temperature limits, except for Papua, New Zealand, and Madagascar. They are probably descended from ancestors common with the Pelobatidae. They probably arose in the eastern portion of Equatoria, late in the Triassic (fig. 232, p. 297), just before the Pelobatidae were evolved and just before the separation of Australia. Some of the family spread westward by the Equatorian route to India, Africa, and South America and the genera characteristic of these regions evolved. Another group of genera arose in Australia. The family seems not to have spread during the Jurassic (fig. 233) from Australia to continental Asia, since no genera other than *Bufo* are known from Asia north of India, and we shall soon see reason to believe that *Bufo* is post-Jurassic in origin.

Bufo (fig. 226) apparently is more modern, having been derived doubtless from some Equatorian form. It is now wholly absent from Australasia, so could not well have been in Antarctica when this continent was connected with Australia. No genera of the Bufonidae other than *Bufo* are known from Asia north of the Himalayas, so *Bufo* probably did not arise in Palearctica. Where did *Bufo* arise? Not in Australasia, for no Bufos are now living in this region. Not in Africa before Madagascar separated from Africa (late Cretaceous, fig. 235, or early Tertiary, fig. 236), for no Bufos are in Madagascar. Not in India, according to Arldt, for fossil Bufos are known from the Oliogooene of Europe, which is earlier than the establishment of connection between the Indian island and continental Asia (figs. 236, 237).

There are two other regions in which there are or have been genera of Bufonidae from which *Bufo* may have been derived. These regions are Malaysia and northern South America. First as to Malaysia. Common ancestors of the Pelobatids and Bufonids, originally eastern Equatorian forms, were probably in Malaysia during the Jurassic (fig. 233). From these, on the north, developed the Pelobatids occupying continental Asia. *Bufo* may have arisen at the same time, moving north into continental Asia along with the Pelobatids. This apparently would have been during the early Cretaceous, just as Australia was breaking away from Malaysia. Bufo may then have moved into all of Asia and into Europe during the

Cretaceous (fig. 234) before the genus *Opalina* was evolved in Asia and to northern South America in the latter part of the same period by way of the Siberia-Alaska-California-Ecuador ridge and have entered North America later (fig. 237), passing to Central America and the West Indies in the later Tertiary.

As to South America. Both *Bufo* and a number of Equatorian genera of Bufonidae are now in northern South America. None of them contain any multinucleated Opalinids, so *Bufo* can not have entered South America from North America and Central America unless it came before *Cepedea* and *Opalina* were in North America and Central America. Apparently *Cepedea* was not in continental Asia until the middle-Tertiary (fig. 237) when Asia and India came together. *Opalina* was probably evolved in Asia from *Cepedea*. *Bufo* may, therefore, have evolved in southeastern Asia during the Cretaceous (fig. 234) and have spread during the same period to South America by the Alaska-California-Ecuador ridge. It could hardly have come to South America by way of North America and Central America, for the Central American route was not open until the later Tertiary (figs. 236, 237) and by that time *Cepedea* and *Opalina* were doubtless present in the North American and Central American toads. If the South American Bufos came from either North America or Asia during the late Tertiary they would now bear *Cepedea* and *Opalina*, but they do not. *Bufo*, therefore, either came to South America in the Cretaceous by the Alaska-Ecuador ridge, or it evolved in northwestern South America, Ecuador, from some genus of Equatorian Bufonid already there, and migrated to Euro-Asia traversing the Ecuador-Alaska ridge in a northward direction. I do not see that our data show which of the two places of origin, Malaysia or northwestern South America, is the more probable.

In either case, *Bufo* apparently did not enter North America directly from South America, for there was no open route by way of Central America until the later Tertiary when *Zelleriella* had reached tropical America, and North American Bufos do not contain *Zelleriella*, the characteristic Opalinid parasite of the South American toads. *Bufo* apparently came to North America from Asia during the Tertiary, traveling by way of Alaska. The Cretaceous Alaska-Ecuador ridge was occupied by Bufos and some of these may also have passed eastward later to middle North America.

Bufo, then, apparently arose in the Cretaceous after Australia had parted from Euro-Asia, evolving either in southeastern Asia or in northwestern South America. It entered North America from the west, not the south. There apparently has been no direct spreading of Bufos from North America to South America or the reverse. *Bufo* seemingly entered tropical Africa from Euro-Asia by way of the Nile valley region in the later Tertiary (fig. 237) after Madagascar had separated from Africa.

Our review of the data as to the Bufonidae and the genus *Bufo* indicates, then, that the Bufonidae are an Equatorian family, dating back to the Triassic period, and that *Bufo* arose during the Cretaceous either in northern South America or in Asia-Malaysia, its origin being two geologic periods, or more, later than the origin of the family. It is worthy of comment that this ancient family has retained its vigor through all the geologic periods since the Triassic and that its perhaps most recently evolved genus, *Bufo*, now disputes with the genus *Rana* the position of dominance among the Anura.

The HYLIDAE (fig. 227, p. 291).

The map showing the present distribution of this family is deceptive, for the great area in Europe, Asia, and Africa, where they occur, denotes the presence of but one species and three closely similar forms, indicating apparently the migration of but one species from North America and its subsequent divergence into four species, or subspecies.

It seems clear that the Hylidae were developed in the Western Hemisphere. They are now found in tropical America north of the region of the former sea which separated the central and the southern portions of South America (fig. 236). They are not known from southern South America and it does not seem probable that they ever were in the Argentina-Patagonia-Chile area. But Hylids are in Australia and Papua, though not in New Zealand. How are these complicated relations to be explained? The moist forests of tropical America (Brazil to Central America) are now the home of the majority of the genera and species of Hylidae. They are peculiarly adapted to forest life. Doubtless they evolved in this region. The grassy plains of Argentina-Patagonia are less suitable for them. If the Hylidae had evolved in tropical South America during the Jurassic, they should have passed to Euro-Asia by the Ecuador-California-Alaska-Siberia ridge (fig. 233), but they did not. Had they evolved in the Guianas during the early Cretaceous (fig. 234) they should have passed eastward to Africa, but they did not. If they evolved in Argentina-Patagonia, this region must at one time have been heavily forested, a thing not in agreement with paleontological evidence, and if they were there, it is remarkable that they are now unrepresented in this area. They are found in North America on the grassy prairies, so it hardly seems likely that change from forest conditions to the pampas should have exterminated them in southern South America. These considerations indicate the region of the Brazilian Highlands as the ancestral home of the Hylidae, and the time of their evolution as Cretaceous or later (fig. 234).

But how did they reach Australia and Papua? They could hardly have taken the Antarctic route without being in Argentina-Patagonia to-day. It looks as if the land bridge across the southern Pacific Ocean from central South America to Australia, which Arldt and Scharff have suggested, must be called in to account for the the presence of the Hylidae in these two regions, and the map of the late Cretaceous has been drawn to express this view, though there is little evidence for placing the trans-Pacific bridge at this time rather than in the early Tertiary (compare Arldt, my figure 236, *A*). The fact that the Australasian forms have not diverged into other genera than *Hyla* is a slight argument in favor of the later connection.

We have already noted that the South American genus *Zelleriella* is the characteristic Opalinid of the Leptodactylidae, whose origin in the Argentina-Patagonia-Chile region will soon be discussed. The fact that no Australian and only three American Hylids harbor *Zelleriella* seems at first thought a further indication that Hylids did not pass from America to Australia by way of Patagonia, the early home of *Zelleriella;* but the force of this suggestion is much lessened when it is noted that since middle Pliocene times Leptodactylids with their Zelleriellas have been in contact with Hylids throughout all tropical America, and of course in Australia also. The Hylids are resistent to infection by Zelleriella and doubtless always have been so. In their migration to Australia the Hylids carried archaic Protoöpalinas but not Zelleriellas.

The passage of Hylids from Australia to Papua could have occurred in the early Tertiary, according to Arldt's charts (fig. 236, *A*). Absence of Hylids from New Zealand suggests New Zealand's isolation at the time of the Australia-Brazil connection.

The Hylidae now occupy all tropical South America and Central America, the Greater Antilles, the Bahamas, and the Bermudas, and have spread over all of North America where climatic conditions allow. Since the middle Pliocene, or possibly a little earlier, the Isthmus of Panama was available for their northward migration (Vaughan, 1919), and about the middle of the Pliocene the two ridges, from Yucatan to Cuba and from Honduras to Jamaica, were open to them. At this time the Bahamas were united to the Greater Antilles. The fauna and flora of the Bermudas present difficult problems, and the presence there of a *Hyla* with a *Zelleriella* is one of the difficult things to explain.

Entrance to Central America from northern South America was apparently possible during the early Tertiary by way of the eastern Pacific land strip (fig. 236). Scharff (1911) has presented considerable evidence of a connection from this land strip to eastern North America across the Sonoran region of northern Mexico and southwestern United States. Hylids may therefore have been in Central America and eastern United States since the earlier Tertiary. This would be in agreement with the fact that these regions show several genera and numerous species not found in South America. This diversification might, however, have occurred since the early Pliocene in the case of such a vigorous family as the Hylidae.

Three (or four?) species, or subspecies, of *Hyla* are known from eastern Asia, Europe, and northern Africa. *Hyla arborea* of Europe carries a species of narrow *Opalina*, *O. obtrigona*, and the same *Opalina* is found in the two subspecies of *Hyla arborea* examined (*savignyi* from Asia Minor and *japonica* from Japan). This is evidence that the Euro-Asian Hylas came from America. This must have been a comparatively recent migration, for it doubtless occurred after the broad Opalinas had passed from Asia to North America in the middle or late Tertiary, had there met and been adopted by the Hylids and had been changed by their new hosts from broad to narrow form. Of course, some might claim that if the Hylids in North America could change a broad *Opalina* into a narrow one, an emigrant *Hyla* in Euro-Asia could do the same thing. Such parallel evolution, however, is not as probable as is the suggestion that the emigrant *Hyla* carried its narrow *Opalina* with it from America.

Note the extent of the spread of the Hylidae: From southeastern Brazil to all tropical America, including Central America, the West Indies, and the Bahamas; to all North America, and on by way of Alaska to Siberia and China and across to Europe and northern Africa; also from tropical America to Australia. A wider spreading would be possible only by adding the distance from southern Brazil to Cape Horn and from Abyssinia to the Cape of Good Hope. This wandering may mostly have occurred since the middle Pliocene when the Isthmus of Panama was established, but of this we can not be certain. Only the Bufonidae, among the Anura, have a wider distribution.

It is noteworthy that migration of Hylids from Central America southward into South America apparently has not occurred, at least not with any freedom, for if it had occurred in any abundance some Hylids bearing species of the genus *Opalina* should be found in South America, since half a dozen Central American species of Hylidae carry *Opalinae;* but no *Opalinae* are known from South America in these or any other hosts.

To summarize: The family Hylidae seems to have arisen in the southeastern Brazilian highlands, from forms related to the early Bufonidae. They spread to Australia by a southern trans-Pacific bridge in the late Cretaceous or early Tertiary. They spread throughout northern South America in the middle Tertiary and either then or in the later Pliocene passed on to Central America and North America. In the later Pliocene they passed to the West Indies and the Bahamas from Central America. Probably during the same period one species passed by way of Alaska to Siberia, China, Europe, and northern Africa, evolving as it went into three (four?) closely related species, one of which has diverged into four (five?) subspecies.

We have noted that the Hylidae, whose original Opalinids were *Protoopalinae*, have been in general inhospitable to the binucleated Zelleriellas, but have accepted some Cepedeas and are chiefly infected with Opalinas. Although originally bearing parasites of a binuclated genus they later were more ready to accept multinucleated guests than binucleated Zelleriellas, and this too in spite of the fact that they have been in contact with Zelleriellas for a considerable period, that is since the early or middle Pliocene.

The LEPTODACTYLIDAE (fig. 228, p. 292).

The Leptodactylidae are very abundant in South America; are numerous in Central America; have barely passed the barrier of the northern Mexican desert, being represented in Texas by two species; have entered the West Indies, but are only sparcely represented there; they are well represented in Australia and Tasmania; one genus, *Liopelma*, with two species, is in New Zeland, being the only Amphibian there; and one species has recently been reported from Papua. *Heleophryne*, of southern Africa, originally classed as a Ranid, has recently been shown to have an arciferous sternum and has been assigned to the Leptodactylidae. Dr. L. Stejneger suggests in a letter to the author that, as this is the youthful condition of the sternum in the Ranidae, *Heleophryne* may be a Ranid with arrested development. The occurrence of a true Leptodactylid in Africa seems so utterly anomalous that I am leaving this form out of account in the discussion of distribution. I venture the prediction that *Heleophryne* will not be accepted finally as a Leptodactylid. As an illustration of the conclusive character of evidence from parasites we may note that were *Zelleriella*, the Opalinid of the Leptodactylidae, known from *Heleophryne* we should have to accept the latter as a Leptodactylid and set ourselves to solve the puzzle of its occurrence in Africa. But there is little likelihood that this South American Opalinid will be found in this African Anuran. The connection between southern Africa and Patagonia was apparently broken too early to serve as a bridge for Leptodactylids.

The Leptodactylidae seem an Antartic family, with much the same history as their cousins the Hylidae. They arose apparently in

Argentina-Patagonia, for they are much more numerous in both genera and species in South America than in Australasia. In southern South America the Leptodactylids evolved their characteristic parasites, the Zelleriellas. These "southern frogs" passed, probably during the early Miocene, to New Zealand, Tasmania, and Australia, and one species has gone on into Papua. In this spreading they gave rise to a number of genera. Northern and southern South America uniting at about the beginning of the Pliocene, the Leptodactylids entered tropical America, becoming there, with the Hylidae, the dominant Anura. Their Zelleriellas are the dominant Opalinidae of tropical America. The Leptodactylids may have migrated westward to Australasia just before New Zealand and Papua became isolated, for they are barely represented in these two regions. It will be of much interest, when the New Zealand and Papuan forms are searched for Opalinids, to see if *Zelleriella* is found. If it is not found, this will be something of an indication that *Zelleriella* developed late, just before the migration of Leptodactylids to Australia ceased. This is the indication from the already known abundance of *Zelleriella* in South America and its comparative scarcity in Australia and Tasmania.

The Leptodactylids carry *Protoopalina* and *Zelleriella*, both binucleated genera. They do not carry any of the multinucleated species, though they have been in contact with *Cepedea* throughout their American range (excepting only the West Indies) and have met both broad and narrow *Opalinae* in Central America. On the other hand they have given their Zelleriellas to the Anuran hosts of these multinucleated Opalinids. The Hylidae, however, in contact with the Leptodactylids since the middle Pliocene, have proven almost completely resistant to *Zelleriella*. The two families Hylidae and Leptodactylidae, having come into contact with all the genera of Opalinidae and nearly all their subgeneric groups of species, have reacted very differently to the several groups of Opalinids, the Leptodactylidae completely rejecting all the multinucleated species and being hospitable to the binucleated forms, while the Hylidae, originally containing *Protoopalina*, reluctantly adopt *Zelleriella* in but three cases, but readily accept the multinucleated forms.

The GASTROPHRYNIDAE (fig. 229, p. 293).

The Gastrophrynidae are a fairly large family. Their species are found in tropical America (one form reaching north into southeastern United States) in tropical and southern Africa, in Madagascar, India, Cochin China, and the East Indies (Papua and an adjacent island). In America their parasites are Protoöpalinas, Zelleriellas, Cepedeas and narrow Opalinas. The absence of Gastrophrynids from Australia is an indication, but not of course conclusive, that they were not present in the Patagonian fauna at the time Patagonia was united to Australia and that the route to Australia, taken by the Brazilian Hylids, was not open to them. It is likely the Gastrophrynids in South America were confined to the Guiana highlands until after routes of migration from South America to Australia had been permanently closed. The Gastrophrynidae seem a fairly vigorous family so that the negative evidence from their absence from Australia deserves emphasis. Their presence in Papua and in an adjacent island just to the west of it is a puzzle, emphasizing again the need of review of all the evidence as to the paleogeographic relations of Papua. The genera in the Eastern Hemisphere are much more numerous than those in America, indicating that probably the family arose in the east.

The Opalinid parasites of the Gastrophrynidae do not throw much, if any, light upon the problem of their distribution. It is in two Central America species of *Gastrophryne* that we find two *Protoopalinae* (*P. ovoidea* and *P. xyster*) which seem transitional forms between the *Protoopalinae* and *Zelleriellae.* It is, however, evident, on the basis of broader data already reviewed, that *Zelleriella* arose in southern South America, before its union with the northern portion of the continent. *Protoopalina xyster*, which by its flattening approaches *Zelleriella*, is found, not in any species which could have been an early inhabitant of Argentina-Patagonia, but in a species from a family not represented in the early southern fauna, a *Gastrophryne*, probably descended from an immigrant from Africa to Guiana. As the toads, and to a less extent the frogs, adopted *Zelleriella* from the Leptodactylids, when these families met after the disappearance of the trans-South American sea, so also the American Gastrophrynids, under similar conditions, secondarily acquired *Zelleriella. Protoopalina xyster* may be a representative of an early group of southern South American species, originally parasitic in Leptodactylids, which were transitional between *Protoopalina* and *Zelleriella.* It is curious, but not at all inexplicable, that our only at present known examples of these transitional forms have survived, not in an original Leptodactylid host, but in a secondarily adopted Gastrophrynid host.

The Gastrophrynidae apparently arose in early Cretaceous times (fig. 234) in South Atlantis, or more probably in its Madagascar-India extension. Persisting in India during the early Tertiary (fig. 236), they entered Asia in the late Tertiary (fig. 237). Their presence in Papuasia is unexplained.

The Ranidae (figs. 230, 231, p. 294).

The Ranidae have their nearest relatives probably in the Gastrophrynidae. The distribution of the two families is very similar (figs. 229, 230, 231), except for the two facts, first, that no Ranids are in the southern half of South America and, second, that the Ranidae have spread throughout Arctogea, being represented in North America and Europe by the genus *Rana* and in northern Asia by the genera *Polypedates* and *Rana*. A fossil form, assigned to the genus *Oxyglossus* (one of the *Raninae*), whose present home is in India and the East Indies, is reported from the "Eocene" (Gadow, 1909) or "Comanchian" (Schuchert, 1915 ?) of Wyoming, but the identification is of very doubtful validity. Both families are well represented in tropical Asia and Africa. Both are in Papua. Both are in South America. Both *Raninae* and *Dendrobatinae* are in tropical Africa, Madagascar, and tropical America, but the *Dendrobatinae* are absent from tropical Asia-Malaysia-Papuasia. Both Ranids and Gastrophrynids are absent from Australia, Tasmania, and New Zealand. Omitting the two northern genera, *Rana* and *Polypedates*, and the Wyoming fossil, the distribution of the two families is seen to be almost identical, the only difference being the presence of a few Ranids in the Malay Islands and their absence from the southern half of South America. Both families seem to have arisen in South Atlantis (Africa-South America) and its Madagascar-India extension in early Cretaceous times (fig. 234) and to have entered continental Asia from India during the late Tertiary, spreading during the same period to Malaysia, Japan, and Europe.

The subfamily *Raninae* has the same distribution as the family, except that it is not in the Solomon Islands. The South American genera should be more thoroughly searched for Opalinids. *Phyllobates* and *Prostherapis* from Venezuela and Columbia bear *Zelleriella*. *Phyllodromus*, *Calosthetis*, and *Hylixalus* have not been examined. The three poorly preserved specimens of the South American *Rana palmipes*, which were examined, showed no Opalinids.

The important genera *Polypedates* ("*Rhacophorus*") and *Rana* deserve further mention. *Polypedates* occurs in Madagascar, Ceylon, India, Malaysia, including the Philippines, and through eastern Asia into Japan. It seems to have arisen in India or upon the Madagascar-India ridge and to have passed to continental Asia and into its present localities during the later Tertiary. (Fig. 237.) Its absence from Africa is unexplained and seems in disagreement with the charts upon which our discussion is based, for they show Madagascar united to Africa after the Madagascar-Ceylon-India bridge had been interrupted.

The genus *Rana* is found in Asia (both south and north of the Himalayas), Japan, Formosa, the Philippines, Malaysia, Papuasia; in Europe and northern Africa; in tropical Africa; in North America and Central America, one species being known from the northern third of the continent of South America; on the tip of the northernmost peninsula of Australia, across from Papua, is a single species of *Rana*, perhaps a stray from Papua. The single South American species seems to be a recent immigrant from the north by way of the Isthmus of Panama, but its Opalinid parasites are not known; when known they may tell us its place of origin and the date of its southward migration. *Rana*, like its near relative *Polypedates*, probably arose in India, or upon the India-Madagascar ridge, at about the time the latter was breaking up, and, with *Polypedates*, entered continental Asia from India in the later Tertiary, spreading to its other present habitats. *Rana*, but not *Polypedates*, passed westward by a route north of the Himalayas to Europe, and passed by way of the Nile valley into tropical Africa. Its spread from India northward and westward to Europe and on to southernmost Africa, and its spread northward and eastward to the whole of North America and on into Central America, one species passing across the Isthmus of Panama and as far as easternmost Brazil, rival the spread of the genus *Hyla* from southeastern Brazil, first, westward to Australasia and, second, northward through all America to Alaska, passing on westward to Asia, Europe, and northern Africa.

The presence of *Rana* in Papuasia, though absent from Australia (except for one species at the northernmost tip of this continent, very likely a stray), like the similar distribution of the Pelobatidae and Gastrophrynidae already noted, is an indication that Papuasia and Malaysia were at some time in union after Papua and Australia had finally separated from one another.

The presence in Wyoming of a fossil form assigned to the genus *Oxyglossus*, one of the *Raninae*, presents an interesting problem. *Oxyglossus* is living to-day in India and Java. It apparently reached Java from India in the late Tertiary (fig. 237) or later. Arldt's charts show no connection between North America and Equatoria, or the land masses into which Equatoria later became divided, at any period between early Jurassic and the late Tertiary. According to our paleontological records the Devonian was the period of the rise of the primitive Amphibia, not, of course, including the Anura. Schuchert (1915?), in mapping the early Permian continents, shows broad connections between Europe and the African region of Equatoria, with wide connection between Europe and North America, and we might possibly conceive the evolution of the Ranidae as having occurred as early as the Permian. The existence of the genus

Oxyglossus in Permian times in India and its spread from India through Africa and Europe to Greenland and on to western North America, and the persistence of the genus in India and Malaysia until to-day would be most remarkable and hardly believable. The fossil form in Wyoming, assigned to the genus *Oxyglossus* is said by Schuchert (1915?) to be of the Comanchian period, though it was earlier assigned to the Eocene. Accepting the idea of land connection between all continents in the Permian and the existence of North Atlantis and South Atlantis both in the early Triassic and the early Jurassic, we must ask why the *Raninae*, if already evolved in any of these periods, did not make more general use of the bridges, and how it happens that only *Oxyglossus* succeeded in crossing from one hemisphere to the other. In this connection, however, we should remember that *Raninae* have lived in northern South America apparently since early Cretaceous times, but have not spread to the rest of that continent.

The presence of *Oxyglossus* in North America at any time would be remarkable, for to-day there are in all Arctogea (omitting Asia south of the Himalayas, and Africa, both originally portions of South Atlantis) no *Raninae* except of the two genera *Rana* and *Polypedates*. It seems difficult to accept the identification of the Wyoming fossil as an *Oxyglossus* or even as a Ranid.

If the Wyoming fossil were a Miocene *Rana* there would be no difficulty, or we might possibly reconcile the presence of a representative of the Gastrophrynidae, but a Comanchian *Oxyglossus* seems in itself most strange and also seems far astray in Wyoming. I make no attempt to resolve the puzzle, but must express doubt of the identification of the fossil as an *Oxyglossus*, and I may be allowed to confess to some doubt of the Comanchian reference of the strata if the fossil is a Ranid of any genus.

The South American genera of *Raninae* (*Phyllobates*, *Prostherapis*, *Phyllodromus*, *Calosthetis*, *Hylixalus*) probably reached northern South America along with the *Dendrobatinae* in the early Cretaceous (fig. 234).

The DENDROBATINAE (fig. 231, p. 295).

The distribution of this subfamily suggests origin in South Atlantis in early Cretaceous times (fig. 234), making its evolution about contemporaneous with that of another branch of the frog family, the *Raninae*.

GENERAL CONSIDERATIONS.

Having in mind now the chief indications as to the dates and places of origin and the dates and routes of the spreading of the several groups of Opalinidae and their Anuran hosts, we may profitably discuss a few further points.

The distribution of the Anura and their Opalinids gives no evidence of direct spreading in either direction across the Atlantic Ocean between Europe and North America. The absence of *Protoopolina* and *Cepedea* from New England is signicant in this connection.

We note that our data agree with the general belief that lines of demarcation between northern and southern areas have had, on the whole, more influence upon migration routes than have separations between eastern and western areas. Spreading has been more along lines of longitude than along lines of latitude. Especially emphasized have been the routes between Australasia and Patagonia; between Australia, India, Africa, and South America; and between North America and Siberia. North and south migration between Australia and Asia has been restricted apparently chiefly to the Jurassic. Since the beginning of Mesozoic times Africa seemingly has had communication with the Euro-Asian lands to the north of it in the early Triassic (India), in the early Jurassic (India) and since the middle of the Tertiary (Isthmus of Suez), but we have little evidence of the passage of Anura between Africa and Euro-Asia except in the later Tertiary. The Isthmus of Panama and Central America as a whole has not been as freely used for migration by the Opalinids and Anura as would seem natural. The *Pipinae*, Leptodactylidae, Gastrophrynidae, *Raninae* and *Dendrobatinae* of South America have not passed northward; the Pelobatidae have not passed from North America, into the southern continent, nor have any of the *Raninae* except the one species *Rana palmipes*, the other genera of *Raninae* present in South America never having been in North America. The *Pipinae* seem to be decadent forms and so are of less significance in this connection, but the Leptodactylidae and Gastrophrynidae are vigorous southern forms. The *Dendrobatinae* and the distinctively South American genera of *Raninae* have not shown much recent ability to spread, being still confined to their old northern South American habitat. The Pelobatidae, on the north, are far from dominant forms, but they have spread widely in North America. *Rana*, *Bufo*, and *Hyla* are the dominant genera of Anura to-day. Why has *Hyla* been able to pass northward, while *Rana* and *Bufo* have not entered South America from the north at least in times since the genus *Opalina* was in America, except for the one species, *Rana palmipes*, which indeed probably entered South America after *Opalina* reached North America? Ranas and Bufos are present in Central America and they bear both *Opalinae latae* and *Opalinae angustae*. It is but a step across the Isthmus into northwestern South America where general conditions are very similar to those in Central America. Why have they not taken this step? Our data seem to give us no solution of this puzzle. The northern Mexican desert has apparently been an important bar to free passage between the two continents, but Hylidae have readily passed it and toads and frogs have in considerable numbers moved across it to tropical Central America. There have been two regions of hindrance, the Isthmus of Panama and the northern Mexican desert. That the latter should bar the passage of moist-skinned forms like most of the Anura seems but natural, but why has the Isthmus been a bar during the recent times when it has not been interrupted?

Temperature conditions can hardly have been the controlling element. The Pelobatidae are known from tropical Malaysia, but have not entered the American Tropics. This may well be due to the Mexican desert holding them back. The Gastrophrynidae thrive in the Southern Temperate Zone, though they prefer the Tropics. The Mexican desert probably has hindered their migration but has not prevented it altogether, for they are represented in southeastern United States. The Leptodactylidae live in both Tropical and Temperate Zones in the South. Their northward movement has probably been prevented only by the Mexican desert. The *Dendrobatinae* are apparently strongly tropical in their preferences, but they might well have moved up to the northern coast of the Gulf of Mexico and into Florida, except for the desert. But no review of the data, group by group, explains why the Central American forms have not passed south into South America. The axis of the Isthmus is high ground and there is something of a coastal plain most of the way on one or both shores, so that there is altitudinal diversity of climate, and the hindrance can not well be climatic.

Among the Opalinidae we find no indication that conditions of temperature, except of course extreme cold, have proven a bar to migration. *Protoopalina* is cosmopolitan (fig. 239), excepting only New Zealand, Madagascar, and southern Asia, and they may be unknown from southern Asia only because our material from this area has been very scant. *Zelleriella* is Antarctic (fig. 248). Its Leptodactylid hosts have not entered North America to any extent, and apparently the toads have not passed from tropical America to North America after meeting and adopting *Zelleriella* in tropical America. *Cepedea* is cosmopolitan, except for Australasia and New Zealand. (Fig. 249.) Neither temperature or other climatic conditions have hindered its spread. It is doubtful whether *Cepedea* has reached South America from the north or only from Africa by the northern trans-Atlantic bridge. Its having entered from Africa seems indicated by a study of the African and South American species (p. 337 *et seq.*). The broad *Opalinae*, Eastern Hemisphere forms, have sent a few migrant species into western North America, and the narrow *Opalinae*, Western Hemisphere forms, have sent one representative into Euro-Asia since the early Pliocene, but while the broad *Opalinae* have entered equatorial Africa, neither broad nor narrow *Opalinae* have passed from North America into South America. Each of the four genera thrive under widely diverse conditions of temperature.

Noting the indications as to the Opalinid and Anuran fauna of the three great land zones, Antarctic, equatorial, and northern, we may say: I, 1, In Equatoria arose; *a*, the earliest Opalinidae, *Protoopalinae*, not later than Triassic times, and in Anura probably more primitive than any now persisting; *b*, the Discoglossidae; *c*, the Bufonidae, excluding the genus *Bufo;* 2, In Australasia, after it separated from Equatoria, or in southeastern Asia-Malaysia with which Australia promptly united, arose groups 2, 3, 4, 5, and 6 of the genus *Protoopalina* and also the Pelobatidae; 3, In South Atlantis (South America-Africa and its Madagascar-India extension, the Jurassic and early Cretaceous continent formed from western Equatoria) arose groups 7 and 9 of the genus *Protoopalina*. the genus *Cepedea* and its divisions 4, 6, and perhaps 5, also the Gastrophrynidae, the *Raninae* and the *Dendrobatinae;* 4, in the Brazilian highlands the Hylidae evolved during the early Cretaceous; II, In Antarctica and connected South American lands arose *Zelleriella* and the Leptodactylidae; III, In Arctogea arose *a*, group 8, of the genus *Protoopalina; b*, division 2, and perhaps also division 5 of the genus *Cepedea; c*, *Opalina* and both its subgeneric groups *Opalinae latae* and *Opalinae angustae; d*, the genus *Ascaphus* among the Discoglossidae; *e*, the genus *Scaphiopus* among the Pelobatidae; *f*, the genus *Acris* and possibly the genus *Chrophilus* among the Hylidae, although the latter genus is known also from northwestern South America.

Anura arising in the western end of the continent Equatoria show remarkable migrations: 1. the Hylidae evolved in the Brazilian highlands during the isolation of this region in the early Cretaceous and, as new connections were established, they spread first to Australia and later throughout tropical America and all Arctogea; 2, the Leptodactylidae evolved in Argentina-Patagonia-Chile during the Cretaceous or early Tertiary separation of this land mass from both tropical America and Africa, and later spread by way of Antarctica to Australia and New Zealand, probably during the early or middle Tertiary. There have thus been in the western hemisphere northward migrations from Equatoria to Arctogea and southward migrations from Equatoria to Antarctica. In the eastern hemisphere there was jurassic migration of Equatorian Anura from Australia to

Asia, and there have been late Tertiary migrations between other originally Equatorian lands and Arctogea—from India northward, and from Euro-Asia southward into Africa.

The paleontological evidence as to the times and places of origin of the Anura and their several groups is very scant, a fact not easy to understand. *Oxyglossus*, one of the *Raninae*, is reported from Wyoming in a deposit whose age is given as Eocene by Gadow (1909), and as Comanchian by Schuchert (1915), but this is very difficult to accept. *Palaeobatrachus*, which seems to be similar to the Pipidae, is reported from the mid-Tertiary from Europe (Gadow, 1909). *Bufo serratus* and *B. gessneri* are reported from Oligocene and Miocene rocks from Europe (Gadow, 1909). The Anuran fossil, *Oxyglossus*, reputed to be the earliest known, is said to be a member of probably the most highly evolved family. It is not surprising, therefore, to find that evidence from the distribution of their characteristic parasites, the Opalinidae, puts the origin of the Anura back at least to the Triassic, two geologic periods earlier than the earliest date claimed for any Anuran fossil.

What groups of the Anura are to-day dominant and what ones seem to be disappearing? This question is of interest in weighing the evidence presented by the distribution of the several groups. The Pipidae seem an archaic family not particularly successful today. The Discoglossidae seem to be disappearing. The Pelobatidae have adopted retiring habits and are persisting, but show no great vigor as conquerors of the earth. The Gastrophrynidae are nowhere dominant now, but they show no special indication of being on the wane. The Leptodactylidae, the Hylidae, the Bufonidae, and the Ranidae are all in full vigor and are important elements in the faunas of their several habitats.

In all this review of the hosts and the geographical distribution of the Opalinidae, we see that there are limitations to the dispersal of the several groups of parasitic species both in the families of their hosts and geographically. We have regarded *Protoopalina* as the most archaic genus of Opalinidae, *Zelleriella* as more modified, *Cepedea* as further modified in another direction, and *Opalina* as the most modified genus of the family, though considered from the standpoint of the inhibition of division, *Cepedea segmentata* would be the most highly evolved species in the family. Viewed in the broad, there is a rather curious parallel between the relative degrees of evolution of the hosts and of their parasites. The Protoopalinas are found in fishes and Urodeles (probably modern infections), and are well represented in the lower families of Anura, being found also in a good many species of the higher Anuran families. The Zelleriellas are not well represented in the lower and the highest families of the Anura, but are abundant in the Bufonidae and the Leptodactylidae and are well represented in the Gastrophrynidae. The Cepedeas are not abundant in the lower families of the Anura, but are very numerous in the Bufonidae and the Ranidae. The Opalinas are very scantily represented in Discoglossidae (one doubtfully normal infection) and Pelobatidae, but are very abundant in Hylidae, Bufonidae, and Ranidae.

How rapid has been the spread of Anura and their Opalinids? This is an interesting question upon which we have but limited data. The Hylidae apparently reached northern South America from Brazil in the early Tertiary. Before the glacial period they had passed through Central America, the Antilles (the West Indies-Central America connection was interrupted after the Pliocene), and North America, and had crossed into Asia. The passage through Alaska to Siberia probably occurred before the first Pleistocene glaciation, and the passage from eastern Asia to western Europe and northern Africa probably took place before this first glaciation or after the last glacial extension, rather than during any of the comparatively cold interglacial intervals. The migration from South America to Central America, if by way of the Isthmus of Panama, took place during the Pliocene, and the further extension to western Europe may well have been within this period. The Leptodactylidae apparently passed through South America and Central America and into the Antilles during the Pliocene, for probably before the close of the Pliocene the connection of the West Indian lands with Yucatan and Honduras had been broken (Vaughan, 1919). The Leptodactylidae may well have evolved and have spread throughout Antarctica and its connected lands, Patagonia, New Zealand, Australia, between the time just before the separation of Australia from Papua and the time when Patagonia separated from Australia. (Fig. 228.)

The spread in South America of the *Raninae*, a most vigorous subfamily, seems not to have been at all rapid. (Fig. 230.) We do not know how much longer they may have been in that continent, but apparently they were there as early as the Jurassic. During the time, however long or short it may be, they have not spread beyond the uplands in the northwestern corner of the continent, except for one species of *Rana*, apparently a recent immigrant, which has passed across the northern regions as far as the extreme eastern point of Brazil. Apparently the northern genera *Polypedates* and especially *Rana* are the vigorous members of the subfamily to-day. The genus *Rana* has apparently spread since the middle Tertiary from India east to Malaysia and Papuasia, north and east through

Asia and North America and on through Central America to easternmost Brazil, and it spread from southeastern Asia by a route north of the Himalayas to westernmost Europe and from Asia Minor south to southernmost Africa.

This all looks as if migration time of the Anura must be reckoned in geologic periods rather than in years, but even a single, short, geologic period, like the Pliocene, is long enough for wide wandering, at least of some families. It is very evident that different families and different genera are very different in the extent to which they spread and in the rapidity with which they spread.

Scharff (1911) writes (p. 552): "It is quite evident that the genus *Tapirus* could not have come across any Bering Strait land connection in Pleistocene times and have traveled to Argentina before the end of the Pleistocene period." This does not seem to me quite so evident. The broad Opalinae evolved during the late Tertiary in southern Asia. After this, Anura, probably *Rana*, bearing broad *Opalinae* traveled during the late Tertiary from southern Asia by way of Siberia and Alaska to America, gave their broad *Opalinae* to some Hylid, which changed the broad *Opalinae* to narrow *Opalinae*. After this evolution of the narrow *Opalinae* in America, a *Hyla* carried them back across the Alaska-Siberia connection and on across all Asia to westernmost Europe and northern Africa (see p. 355). Probably the westward passage of the *Hyla* from Alaska to Siberia occurred before the Glacial period. If so, the Miocene and the Pliocene periods together sufficed for the evolution of one subgenus of *Opalina*, its migration in its hosts to America, the evolution in America of the second subgenus of *Opalina*, and its subsequent migration in its host back across the whole width of Euro-Asia and even on into Africa. This is about as rapid spreading as for *Tapirus* to pass during the Pleistocene from eastern Asia by way of Alaska to Argentina.

Our study of the structure of the Opalinidae and our discussion of the distribution of the four genera and of their species, has led to the conclusion that *Protoopalina* and *Cepedea* are old genera and that *Zelleriella* and *Opalina* were more recently evolved. We have noted also that the two older genera show fairly well demarcated subgeneric groups of species, while the two younger genera do not do so, except that in the genus *Opalina* we can distinguish the eastern forms, *Opalinae latae*, from the Western Hemisphere forms, *Opalinae augustae*. Is it the youth of the younger genera that accounts for their less diversified speciation? It may be. But a broad review of the whole animal kingdom from this point of view would, I think, show that, in many cases, groups destined to become highly diversified may acquire a high degree of diversification soon after their first appearance.

One therefore suspects that *Zelleriella* is to-day a very compact genus, not merely because it is comparatively young, but because stability is in "the nature of the beast."

We have studied the phenomena of geographical distribution of the Anura, using two sets of data, the first set presented by the Anura themselves and the second set given by their Opalinid parasites. It is clear that the two lines of evidence together have many times the value possessed by either set of data alone. For example, it might perhaps be possible, though difficult, to regard the Leptodactylids of Australia and South America as of distinct origin, their resemblance being due to convergence or parallel evolution. Hans Gadow (1909) accepts this general viewpoint in such cases of puzzling geographic distribution.[39] But such a belief becomes impossible in any instance in which we find that the apparently related Anura have closely similar or almost identical parasites. Parallel development, or convergent evolution of both the hosts and their parasites is too large a dose for even the most credulous to accept. It is just this sort of crucial evidence which is presented in certain cases by a concomitant study of the Anura and their Opalinidae. There is great opportunity for the development of similar double or multiple lines of evidence from very many groups of animals and their parasites. To be sure the Anura are particularly favorable when we are considering questions of land connections, for they are terrestrial or fresh-water forms and are unable to endure salt water. *Bufo marinus* is said to be found sometimes in brackish water and even to lay its eggs in such water, but this statement lacks confirmation. Darwin (1875) notes one Indian species which endures salt water, and in the Philippines Pearse (1911) found a species of *Rana* (?) entering salt water in the crab holes on the mud flats, and he also found in several pools tadpoles in all stages up to metamorphosis in water containing up to 2.64 per cent of sodium chloride. In general, as Gadow says (1909, p. 71): "The Amphibia are bound absolutely to the land and to fresh water; transportation across salt water is not excluded, but must be accidental and is not a case of regular 'spreading'." (Also p. 72): "Common salt is poison to the Amphibia; even a solution of 1 per cent prevents the development of their larvae. Consequently seas, salt lakes, and plains incrusted with saline deposits act as most efficient boundaries to normal spreading. * * * Solutions of lime are likewise detrimental to many species, and it is a general fact that limestone terrain is poor in Amphibian life, unless, of course, sufficient accumulation of humus counteracts or prevents the calcareous impregnation of the springs and pools in meadows." We can therefore trust to the full the data as to former land connections which the Anura and their Opalinidae give us.

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[39] "The occurrence of four species of Hylella in South America, one in Australia, and one in New Guinea indicates that this is not a natural genus" (p. 75).

Similar twofold or multifold evidence from other groups is a great desideratum. It is easy to reinforce the evidence from Anura and their parasites by extending the study to other parasites than the Opalinidae. Other Ciliates, *Balantidium* and *Nyctotherus*, are quite abundant in the Anura, so also are Nematodes and Trematodes. Many Flagellates are found, as also are Sporozoa and intestinal Amoebae, though the latter are not well preserved in ordinary museum specimens of Anura. *Discophrya* is another Anuran parasite. A comprehensive review of the parasites of the Anura would definitely settle some questions of former continental connections, and probably all such questions of Cenozoic and Mesozoic land connections will be settled, perhaps, beyond peradventure, when we have similar comprehensive studies of numerous groups of terrestrial animals and their parasites. The data recorded in this paper are but a beginning, but they illustrate a method of approach to these questions which is of supreme value in their solution. I am not delaying publication of the data from the Opalinidae until I can study the other groups of parasites in my material. I hope to be able to turn this material over to special students of these other groups.

The parallel study of hosts and their parasites with reference to geographical distribution and other general problems has been but little used. Von Ihering (1900) in discussing the former separation of South America into distinct northern and southern portions, writes: "Archiplata [the portion of America originally south of the trans-South American sea] contains numerous genera of Mollusca, Crustacea, etc., that are common to Chile and the La Plata district, such as *Unio*, *Chilina*, *Parastacus*, *Aeglea*, etc., including many species *and even their parasites*[40] (Temnocephala), which are identical on both sides of the Andes." Also Kellogg (1905), discussing bird lice, writes: "From this fact of near relationship of hosts in all the cases of parasite species common to several host species it seems almost certain that this common occurrence under circumstances not admitting of migration of parasites from host to host, is due to the persistence of the parasite species unchanged from the time of the common ancestor of the two or more now distinct but closely allied bird species."

Johnston (1912 and especially 1914)[41] makes extensive use of the host-parasite method of approach to several important problems of evolution and of zoögeography. Anyone interested in this method should surely consult Johnston's fine papers.

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[40] Italics mine.

[41] These papers were brought to my attention by Dr. Cort after this volume was ready for the press. I regret that in a former paper (Metcalf, 1920, *b*) I did not refer to Johnston's remarkably interesting use of the host-parasite method of attack upon problems of genetic relationships and paleogeography.

Darling (1920 and 1921) has studied the migrations of human races from data of hookworm distribution, drawing some definite conclusions from these data.

These six publications mentioned illustrate the application of the host-parasite method of study to questions of geographical distribution and phylogeny. The same method can be applied with illuminating results to many other problems. Ancient land connections and separations and the dating of the establishment and loss of such connections; ancient climates; the existence and the dates of former deserts, swamps, forests, mountain ranges, streams, and lakes; the altitude of former land areas; the presence or absence of certain food plants in former times in certain areas; genetic relationship as against parallel or convergent evolution; place and date of origin of families, subfamilies, genera, subgenera and species of both hosts and parasites, and many other questions, may successfully be approached by this method. Tables of the occurrence of parasites and their hosts, similar to the table given in this paper for the Anura and their Opalinidae, might to advantage be compiled for every group of animals and plants, and when prepared would be a mine of information from which to reconstruct Cenozoic geography including faunas and floras. Much light would also be cast upon Mesozoic conditions, and probably much would be learned of Paleozoic conditions by this method.

An illustration of the dating of the origin of a family of animals through the application of the host-parasite method may be given. Gadow (1909) wrote of the "southern frogs," the Leptodactylidae: "Their geographical distribution is suggestive of their being an old family. * * * The overwhelming majority inhabit the Neotropical region, a few forms extending into tropical Central America and into the Antilles; the rest, some 20 species only, are confined to the continent of Australia and to Tasmania." [42] But the evidence we have adduced seems to indicate that the Leptodactylidae (fig. 228) are comparatively modern, having been evolved since Australia separated from Asia, but before Australia and Patagonia separated from Antarctica. That is, they evolved between the early Cretaceous and the beginning of the Pliocene, probably during the midde Tertiary.

The zoögeographical data from the Anura and their Opalinids should be correlated with other zoölogical and geographical (including geologic) data, but this broader discussion may well be in a more general, separate paper, rather than in this volume, which is essentially a study of the Opalinidae. I am, however, introducing here a few maps showing zoogeographical data from other groups, bearing upon two questions often referred to in this paper—namely, the trans-Atlantic bridges uniting tropical Africa with tropical America, and an Antarctic connection between Australia and Patagonia.

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[42] One species is now known from Papua and the New Zealand genus *Liopelma* is now assigned to the Leptodactylidae.

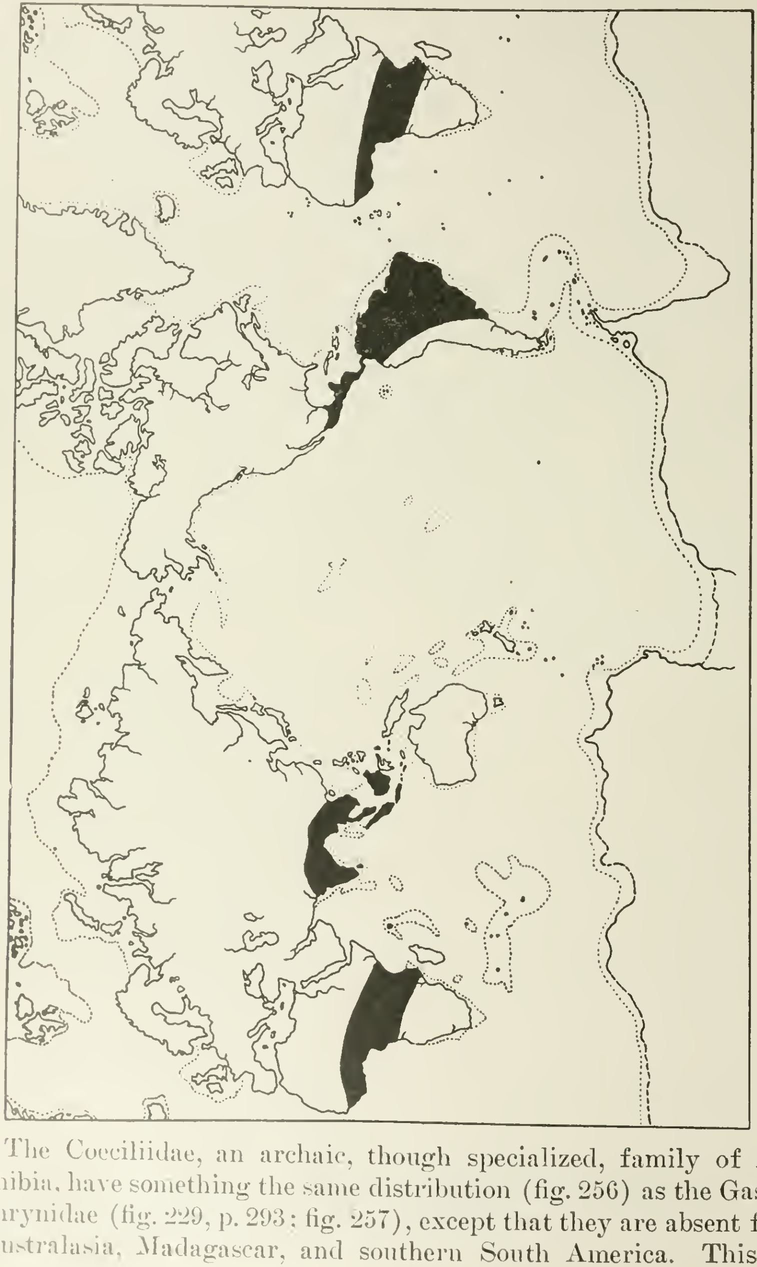

FIG. 256.—THE DISTRIBUTION OF THE COECILIADAE. (AFTER GADOW.)

The Coeciliidae, an archaic, though specialized, family of Amphibia, have something the same distribution (fig. 256) as the Gastrophrynidae (fig. 229, p. 293; fig. 257), except that they are absent from Australasia, Madagascar, and southern South America. This absence from Australasia and southern South America is evidence that they arose north of these Antarctic lands. Their distribution, except for their absence from Madagascar, seems to indicate origin in South Atlantis or its Madagascar-India extension, at some time before the early Cretaceous and a spread to Malaysia during the Tertiary. The absence of Coecilians from Madagascar though they are present in Africa seems explicable only on the hypothesis that they were once in Madagascar and have since disappeared. Their strictly tropical distribution suggests that their present occurrence may, however, be

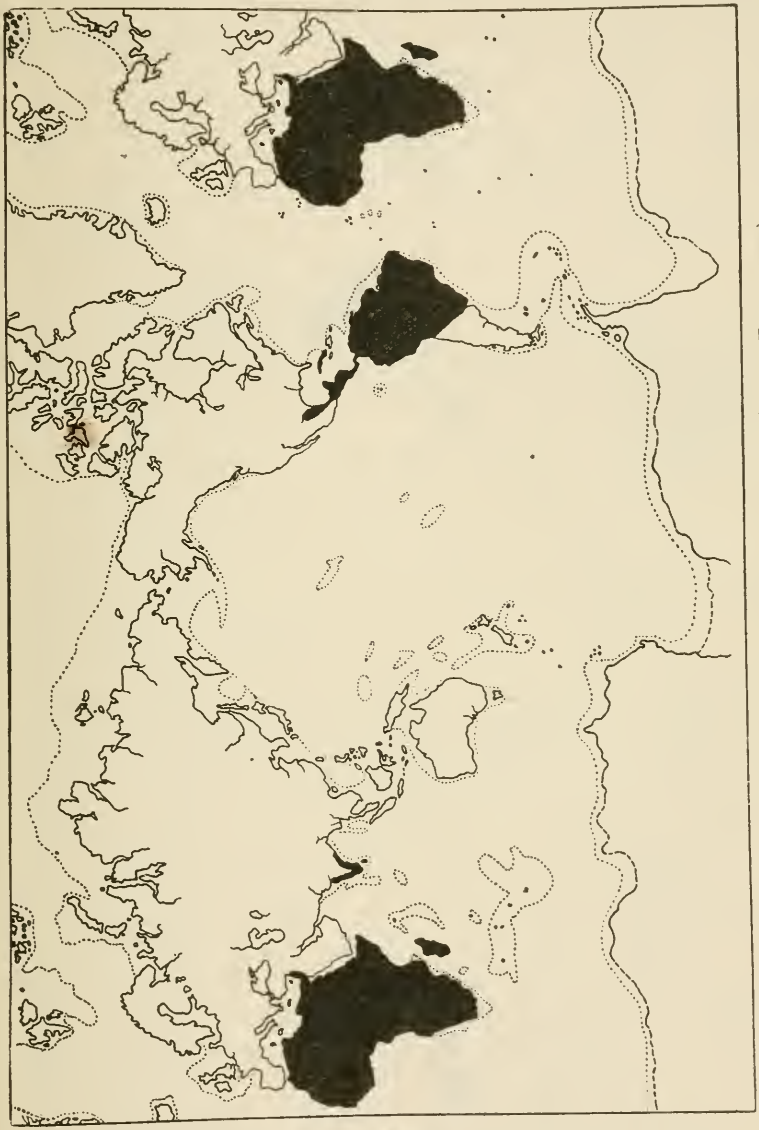

FIG. 257.—THE DISTRIBUTION OF THE CICHLIDAE. (AFTER EIGENMANN.)

due in part to influences other than their place of origin and available migration routes. As we have no fossil remains of this probably waning family, the data from their present distribution deserves less emphasis.

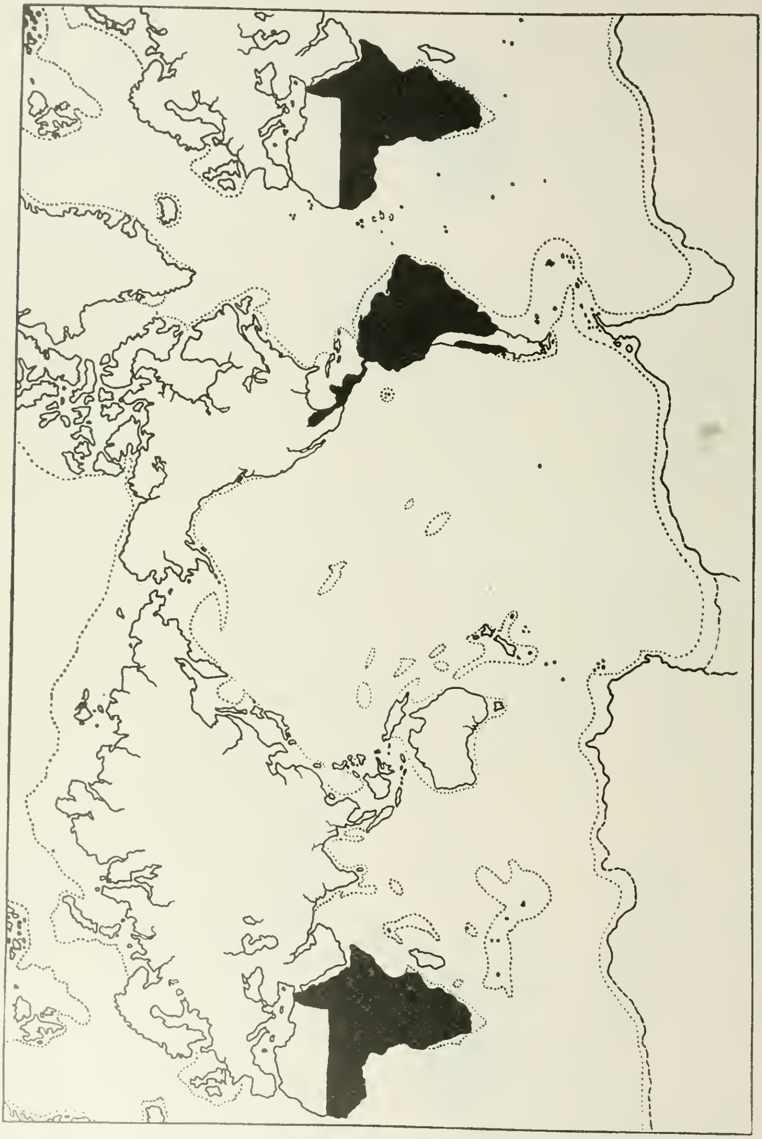

FIG. 258.—THE DISTRIBUTION OF THE CHARACINIDAE. (AFTER EIGENMANN.)

Of two groups of fresh-water fishes Eigenmann (1909) writes:

> The Cichlidae [fig. 257] and Characinidae [fig. 258] are abundant in tropical America and in Africa, a few species of Cichlidae being also found in India. There is no known means by which these two forms could have crossed the existing gap between Africa and South America. There has been no exchange

of species in recent times, for there is no species or genus common to the two continents. The South American and African elements of these two families must have been derived from some intermediate land mass or must have gone from one continent to the other over a land bridge.

As to the Antartic relations of Patagonia we may quote again from Eigenmann (1920). Writing of the fresh-water fishes of the Pacific slope of South America he says:

> One of the questions of interest and importance was where did the fishes of the Pacific slope of South America come from? To answer this question we dipped into many of the rivers emptying into the Pacific from Panama to Puerto Montt. The Irwin expedition has demonstrated that the present condition is not the primitive condition and the present fish fauna is but a relict of the past. Both in Peru and in Central and northern Chile there are great valleys and water courses that must, in the past, have carried great streams. At the present time some of these contain water when the snow melts or when rain falls in the mountains, some are permanently dry. At Piura I asked an urchin where the river was; he answered, "It has not come down yet but we expect it next Saturday." Under such conditions very few fishes have been able to survive. Going south from the extremely wet country of southern Colombia, one species of fish after another drops out until, in northern Chile, nothing is left. Fishes appear again south of Copiapo in Chile, and the first fish is a little thing about 2 inches long, a left over or stray from the Amazon region. Farther south, about Santiago, there is a new lot of fishes that "knew not Brazil," and still farther south, about Concepcion, another group starts in, becoming dominant about Puerto Montt. (Farther I could not go.) This new fauna in southern Chile is more closely related to the fishes of New Zealand than to those of Brazil. They date back to a time when Patagonia was part of a continent separate from northern South America.

The indications involved in the distribution of the Opalinids and their hosts have been stated with but little discussion of geological and other data bearing upon the problems presented. This does not seem the place to enter upon an extended comparative discussion of zoögeography. I am accepting here, for convenience of reference a set of charts mainly from Arldt (1907) but much modified. It is for the zoölogist to develop the zoölogical data, and for the geologist and paleographer to treat critically the geological data. Few new conceptions of geologic phenomena have been presented in this paper. All the land connections and their dating, to which we have referred, have been widely discussed by others; the conditions our maps show in the South American continent and its connections are rather different from prevalent conceptions. The data from Opalinidae and their hosts give new evidence which must be correlated with the evidence from other sources.

In our review of Anuran and Opalinid geography and its implications, we have found general agreement with the conclusions, from much wider data, expressed in Arldt's charts. This statement may well stand as the summary of all our discussion of this subject.

The discrepancies with few exceptions have been slight and inconclusive, while the agreements have been fundamental. We have found our data in agreement with: *a*, the early existence of Equatoria: *b*, an early division of South America into three land masses, Guiana-Brazil on the east, Ecuador on the northwest and Argentina-Patagonia on the south; *c*, a later separation of eastern Brazil from the Guianas and a subsequent union of the Guianas to Ecuador; *d*, a still later union of all these land masses to form the present South American continent; *e*, late formation of the Isthmus of Panama; *f*, connection at a subsequent period between Central America and the West Indies and also between North America and Asia by way of Alaska; *g*, Antarctic connection between Patagonia and Australasia at a time when Patagonia-Argentina was separated from northern South America; and of the disappearance of the Antarctic connection before northern and southern South America united; *h*, an early presence of an equatorial and a southern trans-Atlantic land bridge, of which the southern was the first to disappear; and *i*, connection between Ecuador and Siberia by the Ecuador-Tehuantepec-California-Alaska-Siberia land strip. We have found no evidence of direct trans-Atlantic connection between North America and Europe. The connections of Papua at different periods need further study, and the distribution of the Ranid genera *Polypedates* and *Rana* seems to argue against the conception that Madagascar retained its connection with Africa after it separated from the trans-Indian Ocean ridge. The presence in Wyoming of a Comanchian or Eocene *Oxyglossus* can not apparently be brought into agreement with our charts.

The table on pages 272–285 shows one point of some interest, which it may be well to summarize, namely, the number of hosts and the degrees of relationship between the hosts from which each of the several species and subspecies of Opalinids have been reported. There are listed below the species of Opalinidae which are known from more than one host. *Protoopalina axonucleata* from *Bufo bufo asiaticus*, and its form *lata* from *Rana nigromaculata; Protoopalina caudata* from *Bombina bombina, B. pachypa, Bufo calamita, B. mauritanicus*, and *Bufo viridis; Protoopalina intestinalis* from *Bombina bombina, B. pachypa, Discoglossus pictus* (?) *Pelobates cultripes* (?), *Rana esculenta, Uperoleia marmorata* (?), *Hyla aurea* (?), *H. ewingii* (?), and *Triturus vulgaris* [*Triton taeniatus*]*; Protoopalina tenuis* from *Crinia signifera* and *Uperoleia marmorata; Zelleriella antillensis* is reported from eight species of hosts belonging to two families, but the specific identity of these Zelleriellas is improbable: *Zelleriella dendrobatidis* from *Dendrobates tinctorius* and *D. typographus; Zelleriella engystomopsis* from *Engystomops* *stentor* and *E. pustulosus; Zelleriella intermedia* from *Bufo intermedius* and *B. valliceps; Zelleriella leptodactyli* from *Leptodactylus albilabris*, *L. caliginosus*, *L. gracilis*, and *L. microtis; Zelleriella paludicolae* from *Paludicola bibronii* and *P. brachyops; Cepedea cantabrigenesis* from *Rana cantabrigensis* and its subspecies *latiremis; Cepedea dimidiata* from *Bufo bufo*, *Rana esculenta*, *R. temporaria*, and *R. viridis; Opalina chorophili* from *Choropilus triseriatus*, *C. occidentalis*, *C. ornatus* and *C. feriarum; Opalina copei* from *Rana copei* and *R. pretiosa; Opalina discophrya* from *Bufo cognatus* and *B. copei; Opalina japonica* from *Rana japonica* and probably the same *Opalina* from *R. limnocharis; Opalina obtrigona* from *Hyla arborea* and its subspecies *japonica* and *savignyi; Opalina obtrigonoidea* from *Bufo fowleri*, *B. punctatus*, *Scaphiopus solitarius*, *Hyla femoralis*, *H. arenicolor*, *H. pickeringii*, *Gastrophryne carolinensis*, *Rana pipiens*, and *R. palustris; Opalina obtrigonoidea lata* from *Rana aurora* and *R. aesopus; Opalina obtrigonoidea maxima* from *Bufo boreas* and *B. halophilus; Opalina ranarum* from *Bombina bombina*, *Bufo viridis*, *B. bufo*, *Rana arvalis*, *R. dalmatina*, *R. esculenta*, *R. temporaria* and its subspecies *parvipalmata*, and from *Triturus alpestris* (quite likely a transient infection due to this species devouring tadpoles of *Rana temporaria*); *Opalina virguloidea* from *Hyla eximia*, *H. regilla*, *Rana boylei*, and *R. sylvatica*.

The great majority of the species of Opalinid are known from but one species of host. Several species of Opalinid are known from two or more species of a single genus of host. Six species of Opalinid are reported from hosts belonging to different families of Anura. These cases deserve scrutiny.

Protoopalina caudata is reported from two species of *Bombina* and from a *Bufo*, but the report from *Bufo* is based upon but four specimens of the host from a single locality and these infections may have been but temporary, like the cross infections of European Anura with unaccustomed species of Opalinid, which I easily obtained some dozen years ago. This suggestion, however, does not seem probable in the present instance.

Protoopalina intestinalis is reported from two species of *Bombina*, from *Discoglossus*, *Pelobates*, *Rana*, two species of *Hyla*, from *Uperoleia*, and from the Urodele *Triturus* [*Triton*]. All of these reports except from *Bombina* are somewhat doubtful. The reports from *Discoglossus* are old reports and may likely not be based upon the most careful discrimination. This seems more probable since I have found in *Discoglossus pictus* (the host in question) a *Protoopalina* which I have classed as a subspecies of *P. caudata*, but which might almost as well have been given specific rank. This form might readily be mistaken for *Protoopalina intestinalis* if not closely observed. The original report from *Rana esculenta* I have not found. It is an old report. If correct in its identification of the Opalinid, the infection may perhaps have been but temporary. It is easy experimentally to infect temporarily tadpoles of almost any of the European species of Anura with cysts of almost any European species of Opalinid (see Metcalf, 1909 and Brumpt, 1915). There is, however, a recent report (Andre, 1913) of this species in a Swiss *R. esculenta.* The infection of *Triturus* may well have been to its habit of eating tadpoles of Anura, though the apparently permanent infections of the American Urodele *Ambystoma tigrinum* by *Protoopalina mitotica*, found by Powers, makes the normal infection of *Triturus* by *Protoopalina intestinalis* seem less improbable. The reports from Australia of *Protoopalina intestinalis* in two species of *Hyla* and in *Uperoleia* may very likely be based upon one or more species similar to *P. intestinalis* but really distinct. It would indeed be remarkable to find in these hosts and in Australia true *P. intestinalis*, but we have already noted indications of the long persistence of Opalinids unmodified in their secluded and uniform habitat (p. 356 *et seq.*). We can not, then, with entire confidence accept *Protoopalina intestinalis* as a species which is regularly found in hosts belonging to different families, though this may well prove to be true.

No species of *Zelleriella* is reported from more than one genus of host, except for the report of "*Z. antilliensis* or very similar forms" from five genera of hosts belonging to two families. Probably more careful scrutiny would show several species of *Zelleriella* to be involved. Of the multinucleated forms, *Cepedea dimidiata* is unquestionably found normally in *Rana* and *Bufo. Opalina obtrigonoidea*, or at least forms which in my study I am unable to distinguish from this species, are found in two species of *Bufo*, three species of *Hyla*, a *Scaphiopus*, a *Gastrophryne*, and in two species of *Rana* which are closely similar to one another. It is, of course, possible that in some instances the forms in the different families of hosts belong to distinct species which are so similar anatomically that they can be distinguished only by experiment, but there seems no reason for thinking this to be probable. *Opalina ranarum* is found in *Bombina* (rare), in two species of *Bufo*, and in four species of *Rana*, three of which are closely similar. *Opalina virguloidea* is found in two species of *Hyla* and in two species of *Rana*, which are similar to one another.

We thus see that two species of *Opalinae angustae* and one species of *Opalinae latae* are each found in hosts belonging to different families. The same is true of one species of *Cepedea.* In contrast to these multinucleated forms, we find among the binucleated Opalinids no *Zelleriella* (omitting doubtful identifications) which is known from more than one genus of host, and only two species of *Protoopalina.* In both these cases of *Protoopalina* it seems possible that the reports are based upon insufficient scrutiny in identification or upon temporary infections. If, after further observation, the several binucleated species of Opalinid prove not to be normally parasitic in hosts belonging to more than one family, we have a strange condition of a wider range of infection by single species among the multinucleated Opalinids than among the more archaic Protoöpalinas The point is of sufficient interest to call for further observation.

8. THE OPALINID PARASITES AND THE GEOGRAPHICAL LOCALITIES OF THE SEVERAL SPECIES OF HOSTS KNOWN TO BE INFECTED BY OPALINIDS.

In the last chapter we have looked at the host-parasite relation chiefly from the point of view of the parasites. We should also give the data from the standpoint of the hosts. There follows a tabular statement giving the name of each host whose parasites were studied, the place and date of its collection, the name of the collector, usually the museum catalogue number of the preserved specimen, and the species of Opalinid parasite found. Some additional items may also be given. Most of the hosts studied were museum specimens which had lain, often for many years,[43] upon the shelves of the United States National Museum. If the specimens had been preserved originally in alcohol and had been kept in this fluid, their parasites were generally well preserved, even in some cases in which the alcohol had become weak and the tissues of the host had softened. Specimens preserved in formalin, on the other hand, rarely yielded Opalinids or other intestinal parasites, even though the specimens had later been placed in alcohol. No data as to methods of preservation are given usually upon the Museum records, so this feature is seldom mentioned in the table. It is readily seen that negative records mean little under these conditions, but still it seems best to include them. To distinguish at a glance the positive from the negative data, an asterisk is placed before the record of each host in which Opalinids were found.

Attention is called to the fact that the number immediately following the name of the host species is, in each instance in which it is found, the United States National Museum catalogue number.

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[43] Some specimens that had been preserved for 85 years were found to contain Opalinids and other Ciliata well preserved. Intestinal worms, also, were found in quite good condition.

PISCES.

**Box boops* Linnaeus, Mediterranean Sea, French coast, Leger and Duboscq report abundant *Protoopalina saturnalis.* All adults observed were infected; three-fifths of the small fish were infected.

*Mediterranean Sea, Naples, Italy, from Naples Zoological Station. In many individuals the author found *Protoopalina saturnalis.*

AMPHIBIA.

URODELA.

Family SALAMANDRIDAE.

Subfamily DESMOGNATHINAE.

Desmognathus fuscus (Rafinesque), 5 living specimens, Raleigh, North Carolina; H. H. and C. S. Brimley; no Opalinids.

Subfamily PLETHODONTINAE.

Eurycea rubra (Sonnini), 6 living specimens; Raleigh, North Carolina; H. H. and C. S. Brimley; no Opalinids.

Plethodon glutinosus (Green), 3 living specimens; Raleigh, North Carolina; H. H. and C. S. Brimley; no Opalinids.

Subfamily AMBYSTOMINAE.

Ambystoma opacum (Gravenhorst), several living specimens from Raleigh, North Carolina; H. H. and C. S. Brimley; no Opalinids.

A. maculatum (Shaw) [*punctatum*], 5 living specimens; Raleigh, North Carolina; H. H. and C. S. Brimley; no Opalinids.

**A. tigrinum* (Green), Lincoln, Nebraska, J. H. Powers found abundant *Protoopalina mitotica.*

Two living specimens, Oberlin, Ohio; April, 1910; M. M. Metcalf; no Opalinids.

Five living specimens, Raleigh, North Carolina; H. H. and C. S. Brimley; no Opalinids.

Subfamily SALAMANDRINAE.

Notophthalmus viridescens (Rafinesque) [*Diemictylis viridescens*], 3 living specimens, Baltimore, Maryland; October 29, 1919; M. M. Metcalf; no Opalinids.

Three living specimens, Raleigh, North Carolina; April, 1915; H. H. and C. S. Brimley; no Opalinids. The animals were dead a short time before they were opened.

Ten living specimens, Wood's Hole, Massachusetts; June 25, 1919; M. M. Metcalf; no Opalinids.

* *Triturus* [*Triton*] *alpestris.* Galli-Valerio (1907) reports *Opalina ranarum*, from the Swiss Canton Vaud.

* *Triturus vulgaris* (Linnaeus) [*Triton taeniatus*], Europe. Several observers report *Protoopalina intestinalis.* It is desirable to restudy the Opalinids from this host in view of our present fuller knowledge of the Protoöpalinas.

ANURA.

Family PIPIDAE.

Subfamily PIPINAE.

Pipa pipa (Linnaeus), 3 specimens, United States National Museum No. 57571, 135 mm. long; female, British Guiana, 57572, 135 mm. long; male, same locality, Julius Hurter, Sr., and 39288, 135 mm. long; female with eggs 5 mm. in diameter, T. Barbour; all uninfected.

Nine specimens from the American Museum of Natural History, all from British Guiana, no Ciliata.

Subfamily XENOPODINAE [DACTYLETHRINAE.]

Xenopus calcaratus Buchholz and Peters, 20 specimens, American Museum of Natural History (all collected by the American Museum Congo Expedition), as follows:

* No. 9753, 42 mm. long; female with eggs; Niapu, Belgian Congo; January, 1914; a very few *Protoopalina xenopodos.*

* No. 9705, 43 mm. long; female with about half the usual number of eggs; same label; many *Protoopalina xenopodos.*

Nos. 9705, 9707, 44 and 33 mm. long; same label; many elongated, slender *Nyctotherus;* no Opalinids.

No. 9648, 37 mm. long; same label; *Balantidium;* no Opalinids.

No. 9697, 43 mm. long; same label; no Ciliates.

* No. 9791, 43 mm. long; female with eggs mostly laid, Avakubi, Belgian Congo; January, 1914; elongated *Nyctotherus*, also numerous *Protoopalina xenopodos.*

* No. 9803, 28 mm long; same label; rather numerous *Protoopalina xenopodos.*

* No. 9806, 36 mm. long; same label; apparently a few *Protoopalina xenopodos*, but too poorly preserved for certainty (formalin?).

No. 9792, (36 mm. long), 9797 (30 mm. long), 9799 (44 mm. long), 9804 (31 mm. long) ; same label; elongated *Nyctotherus;* no Opalinids.

No. 9796, 26 mm. long; same label; poorly preserved, nothing found.

No. 9807, 38 mm. long; female with eggs; same label; small Trematodes; no Opalinids.

* No. 9750, 43 mm. long; female with eggs, Medje, Belgian Congo; June 6, 1910; some small, elongated *Nyctotherus*, numerous *Protoopalina xenopodos.*

No. 9751, 43 mm. long; female with eggs; same label; many elongated *Nyctotherus;* no Opalinids.

No. 9650, 37 mm. long; Niapu, Belgian Congo; January, 1910; elongated *Nyctotherus;* no Opalinids.

No. 9762, 41 mm. long; female with eggs; Medje, Belgian Congo; June, 1914; elongated *Nyctotherus*, no Opalinids.

No. 9812, 40 mm. long; Avakubi, Belgian Congo; February, 1914; very stiff from strong alcohol; no Ciliata found.

Xenopus laevis (Daudin), 22 specimens, United States National Museum. Nos. 40690 (48 mm. long), 40716, 40717, 40720, 40722 to 40725, 40960 (90 mm. long), 40908 (52 mm. long), 40910, 40912 (46 mm. long), 41296 (33 mm. long) 41680, 41681, 41683, 41684, 41712 (85 mm. long, female with immature eggs), 41713 (3 specimens, tadpoles from the pouch of a Rosy Pelican, identification uncertain) ; all from British East Africa; no Opalinids.

Xenopus mülleri Peters, No. 19774, 58090 to 58092 (26 and 27 mm. long); all from British East Africa; no Opalinids.

Family DISCOGLOSSIDAE.

* *Alytes obstretricans* (Laurenti), No. 37194, 39 mm. long; female with white eggs 3 mm. in diameter, Central France; a few *Cepedea minor.*

* Collin (1913) reports cysts of some Opalinid from this host in Montpellier, France.

Ascaphus truei Stejneger No. 45632, 52 mm. long; Craigy Pass; 5,000 feet altitude, Siskiyou Mountains, California; September 26, 1909; N. Hollister; no Opalinids.

* *Bombina bombina* (Linnaeus), many specimens from southern Germany, usually infected with *Protoopalina caudata* or *P. intestinalis.*

* Galli-Valerio (1907) reports *Opalina ranarum,* from the Swiss Canton Vaud.

* *B. orientalis* (Boulenger), No. 62346 to 52351, each 48 mm. long; Yulu River, southern Manchuria, China; A. de C. Sowerby; 4 abundantly, 2 scantily, infected with *Protoopalina orientalis.*

No. 17522, 42 mm. long; Fusan, Korea; 1885; P. L. Jouy; no Opalinids.

* Nos 17524, 17527, 17530, each 42 mm. long; Fusan, Korea; 1885; P. L. Jouy; each well infected with *Protoopalina orientalis.*

* No. 17529, 42 mm. long; female with eggs; Fusan, Korea; 1885; P. L. Jouy; abundant infection of *Protoopalina macrocaudata.*

* *B. pachypa* (Bonaparte), many specimens, mostly from Austria, usually infected with *Protoopalina caudata* or *P. intestinalis.*

* *Discoglossus pictus* Otth, No 10052, 1 individual 51 mm. long, 2 individuals 64 mm. long; Sardinia; all heavily infected with *Protoopalina caudata discoglossi.*

* No. 37193, 58 mm. long; eastern Algeria; May, 1893; F. Werner; a few *Protoopalina caudata discoglossi.*

* Brumpt (1915) reports *Protoopalina intestinalis* [?] and *Opalina ranarum* localities not mentioned.

Family PELOBATIDAE

* *Megalophrys montana* Wagler, No. 38955, 58 mm. long.; Tiibodas, Java; 4,500 feet altitude, T. Barbour, some *Protoopalina montana.*

Nos. 44150, 44151, Mount Gede, Java; April, 1909; Bryant expedition; no Opalinids (apparently originally preserved in formalin).

Nos. 44148, 44149, Mount Salok, Java; 3,000 feet altitude, May, 1909; Bryant expedition; no Opalinids (formalin?).

* *Pelobates cultripes* (Cuvier), Collin (1913) reports *Protoopalina intestinalis* from near Montpellier, France.

* *P. fuscus* (Laurenti), No. 37191, 54 mm. long; Klosterneuburg, Austria; May, 1897; F. Werner; a few *Protoopalina pelobatidis.*

No. 11066, 2 specimens, 38 and 50 mm. long; Turin, Italy; no Opalinids (poorly preserved, soft).

Nos. 16448, 16449, 50 and 54 mm. long; "Europe"; no Opalinids.

*No. 16450, 48 mm. long; "Europe"; a few small *Protoopalina pelobatidis.*

Pelodytes punctatus (Daudin), No. 37192, 48 mm. long; Montpellier, France; 1889; T. V. Fisher; no Opalinids.

* *Scaphiopus albus* Garman, No. 52403, 54 mm. long; Key West, Florida; A. Garman; many *Cepedea floridensis.*

* *S. bombifrons* Cope, No. 14554, 51 mm. long; Black Foot Fork, Idaho; Hayden; many *Protoopalina scaphiopodos.*

* No. 22265, 38 mm. long; Los Animas, Colorado; July 18, 1892; A. K. Fisher; very many *Protoopalina scaphiopodos.*

No. 20963, 54 mm. long; Helena, Montana; J. A. Eslik; no Opalinids.

No. 38052, 51 mm. long; Albuquerque, New Mexico; May 31, 1907; J. Hurter; no Opalinids.

* *S. couchii* Baird, No. 16329, 2 specimens, 41 and 60 mm. long; Helotes, Texas; November 30, 1883; G. W. Marnock; both sparsely infected with *Zelleriella couchii.*

No. 52299, Brownsville, Texas; R. T. Camp; no Opalinids.

No. 21706, Waco, Texas; May 14, 1894; H. H. and C. S. Brimley; no Opalinids.

Nos. 38359, 38360, 54 and 57 mm. long; Cameron County, Texas; May 15, 1908; J. Hurter. The larger specimen was a female which had recently discharged eggs; no Opalinids.

No. 12650, 3 specimens, 32 and 51 mm. long; La Paz, California; L. Belding; no Opalinids (poorly preserved).

S. dugesi Brocchi, 16203 to 16207, 5 specimens; 38 to 51 mm. long; Guanajuato, Mexico; A. Dugés; no Opalinids (poorly preserved, soft).

* *S. hammondii* Baird, No. 36365, 64 mm. long; female with eggs 1 mm. in diameter; Beaver City, Utah; May 24, 1905; G. P. Engelhardt; abundant *Protoopalina hammondii.*

* No. 9915, 45 mm. long; Guanajuato, Mexico; 1877; Dugés, many *Protoopalina hammondii.*

* No. 52157, 26 mm. long; Wyoming; Copper; good infection of *Protoopalina hammondii.*

* No. 8327, 32 mm. long; Chihuahua, Mexico; Potts; rather few *Protoopalina hammondii.*

* No. 52149, 19 mm. long; Springerville, Arizona; August, 1914; J. S. Ligon; a very few *Protoopalina hammondii.*

*Nos. 52144, 52147, each 19 mm. long; Springerville, Arizona; August, 1914; J. S. Ligon; one scant, one good, infection of *Opalina oblanceolata.*

*No. 8327, 45 mm. long; female, with eggs 1 mm. in diameter; Chihuahua, Mexico; Potts; very many *Opalina oblanceolata.*

No. 8327, 3 specimens 42 to 64 mm. long; Chihuahua, Mexico; Potts; no Opalinids.

No. 18786, 45 mm. long; Owen Lake, California; May 18, 1881; Stephens; no Opalinids.

Nos. 40211, 40212, each 45 mm. long; Rifle, California, August 15, 1907; M. Cary; no Opalinids.

* *S. multiplicatus* Cope, No. 14599, 32 mm. long: Mexico; September 1: Dugés, very many *Protoopalina mexicana.*

* No. 14599, 32 mm. long; Mexico; September 1; Dugés, no Opalinids.

No. 26597, 54 mm. long; Chihuahua, Mexico; July 18, 1898; H. H. and C. S. Brimley; no Opalinids.

No. 17437, 38 mm. long; Silao, northern Mexico; A. Dugés; no Opalinids (in poor condition).

* *S. solitarius* Holbrook, 3 living specimens, Raleigh, North Carolina; H. H. and C. S. Brimley; all infected with *Opalina obtrigonoidea.*

Same label, one specimen uninfected.

* A. C. Stokes reports *Cepedea* [?] *flava*, no locality or date.

Family HYLIDAE.

*_Acris gryllus_ LeConte, No. 3567, 4 specimens; 22 to 25 mm. long; Salem, North Carolina; Lineback; good infections of _Opalina virguloidea magninucleata_.

*11 living specimens; Raleigh, North Carolina; April, 1915; H. H. and C. S. Brimley; 7 had abundant _Opalina virguloidea magninucleata_, 4 had no Opalinids, 6 had _Nyctotherus_, 4 had Flagellates.

*No. 21385, 32 mm. long; female, with eggs; Creighton, Nebraska; Evermann; many _Opalina virgnboidea magninucleata_.

*Nos. 26472, 26474, each 24 mm. long; Dr. E. A. Mearns; a few _Opalina virguloidea magninucleata_.

No. 26573, 224 mm. long; Dr. E. A. Mearns; no Opalinids.

Agalychnis callidryas Cope, No. 42271, 44 mm. long; Vera Cruz, Mexico; C. R. Orcutt; no Opalinids (formalin ?).

*_A. helenae_ Cope, No. 14186, 4 mm. long; Nicaragua; August 7, 1885; T. R. Bransford; a few _Opalina helenae_.

*No. 19959, 65 mm. long; female, with pale eggs 1¾ mm. in diameter; San Carlos, Costa Rica; Burgdorf and Schild; very many _Opalina helenae_.

No. 16146, 48 mm. long; female, no eggs, enlarged oviducts; no Opalinids.

*_A. moreletei_ (Dumeril), No. 12768, 58 mm. long; Guatemala, "British Museum"; a few _Opalina moreletei_.

*Nos. 24827, 65 mm. long; Guatemala; H. Hogue; a few _Opalina moreletei_.

No. 24828, 24829, 75 and 57 mm. long; Guatemala; H. Hogue; no Opalinids.

*_Chorophilus feriarum_ (Baird), Nos. 15263, 15268, 15269, 25 and 23 mm. long; District of Columbia, United States of America; Henshaw; _Opalina chorophili_.

No. 15266, 24 mm. long; same label; no Opalinids.

*9 living specimens; Raleigh, North Carolina; April, 1915; H. H. and C. S. Brimley; 6 uninfected, 3 had _Opalina chrophili_, two of these had also _Nyctotherus_.

*No. 19631, 29 mm. long; Raleigh, North Carolina; H. H. and C. S. Brimley; _Opalina chorophili_.

No. 19632, 35 mm. long; same label; no Opalinids.

No. 16629, 29 mm. long; same label; no Opalinids.

Nos. 37979, 37980, 30 and 28 mm. long; Olney, Illinois; Ridgway; no Opalinids; poorly preserved.

C. miliaris (Spix), No. 38936, 53 mm. long; Brazil; T. Barbour; no Opalinids.

*_C. occidentalis_ (Baird and Girard), No. 29189, 32 mm. long; Hastings, Florida; June, 1901; Brimley; _Opalina chorophili_, also _Nyctotherus_ and _Balantidium_.

*_C. ornatus_ (Holbrook), No. 13634, 38 mm. long; Texas; November 30, 1833; G. W. Marnock; _Opalina chorophili_.

*No. 15566, 35 mm. long; Cook County, Texas; G. H. Roysdale; many _Opalina chorophili_.

*_C. triseriatus_ (Wild), many living specimens from Raleigh, North Carolina; Baltimore, Maryland; and Oberlin, Ohio; mostly well infected with _Opalina chorophili_.

*_Hyla adlelaidensis_ (Gray), No. 59951, 39 mm. long; Margaret River, Australia; from Museum and Art Gallery of Western Australia; abundant _Protoopalina adelaidensis_.

**H. albomarginata* (Spix), No. 48856, 55 mm. long, female, ovary small, eggs young; Bonito, Brazil; Branner; rather many *Cepedea multiformis*.

*Nos. 14191, 14192, 32 and 30 mm. long; Nicaragua; Bransford; *Opalina multiformis*.

No. 14190, 38 mm. long; Nicaragua; Bransford; no Opalinids.

**H. andersoni* × *H. pickeringii* (?); in a single living specimen of this hybrid (?) tree frog, from Oberlin, Ohio, were numerous *Opalina obtrigonoidea*.

**H. arborea*. Numerous European specimens of this tree frog have yielded to many observers and to the author *Opalina obtrigona*.

**H. arborea japonica* (Schlegel), No. 23907, 32 mm. long; Onogami, Japan; I. Ijima; a few *Opalina obtrigona*.

No. 23908, same label; no Opalinids.

No. 17511, 25 mm. long; Fusan, Korea; 1885; Jouy; no Opalinids.

Nos. 30742, 30743, 28 and 25 mm. long; Miyazaki, Kiusiu, Japan; C. A. Clark; no Opalinids.

Nos. 31915 and 31920, 30 and 29 mm. long; Shikoku, Japan; May 11; H. M. Smith; no Opalinids.

*No. 52354, 35 mm. long; Yulu River, southern Manchuria, China; A. de C. Sowerby; one small bunch of *Opalinae* seen, but not identified.

H. arborea meridionalis (Boettger), No. 37189, 42 mm. long; Oran, western Algeria; 1890; Werner; no Opalinids.

**H. arborea savignyi* (Audouin), No. 37190, 40 mm. long; Jerusalem; Werner; many *Opalina obtrigona*.

H. arborea stepheni (Boulenger), Nos. 21211, 21212, 28 and 18 mm. long; Korea; 1883; Jones; no Opalinids.

**H. arenicolor* (Cope), No. 19736, 46 mm. long; female with eggs; Fort Huachuca, Arizona; 1892; T. E. Wilcox; many *Opalina obtrigonoidea*.

No. 26156, Guanajuato, Mexico; A. Dugés; no Opalinids.

No. 8656, 48 mm. long; Utah; 1872; Dr. H. C. Vanow; no Opalinids.

No. 15716, 48 mm. long; Prescott, Arizona; Capt. W. L. Carpenter; no Opalinids.

**H. aurea* (Lesson), Australia; Raff reports *Protoopalina hylarum*.

*No. 15478, 67 mm. long; Wollongong, Illawara, New South Wales, Australia; January, 1840; United States exploring expedition; myriads of *Protoopalina australis*.

No. 58061, King George Sound, Australia; 1906; Julius Hurter, sr.; no Opalinids.

*Cleland and Johnston (1910) report "*Opalina* sp." [doubtless a *Protoopalina*] from this species of host from Queensland, Australia.

* *H. baudinii* Duméril and Bibron, No. 30410, 53 mm. long; Cordova, Mexico; F. Sumichrast; very many *Cepedea baudinii*.

*No. 24817, 56 mm. long; Guatemala; H. Hogue; *Cepedea baudinii*.

* No. 24819, 47 mm. long; same label; *Cepedea baudinii*, *Opalina guatemalae*, also *Balantidium*.

* No. 24823, 50 mm. long; same label; *Cepedea baudinii*.

No. 24818, 58 mm. long; female with eggs; same label; no Opalinids.

No. 16563, 65 mm. long; Orizaba, Mexico; Sumichrast; no Opalinids (poorly preserved).

No. 25137, 58 mm. long; female with eggs; Sacluc, Guatemala; Berendt; no Opalinids (poorly preserved, soft).

**H. caerulea*, Cleland and Johnston (1910) report "*Opalina* sp." [doubtless a *Protoopalina*] from Queensland, Australia.

H. chinensis Guenther, No. 35529, 38 mm. long; Shensi, China; E. Blackwelder; no Opalinids.

* *H. cinerea* Schneider, No. 51508, 30 mm. long; Victoria County, Texas; February 26, 1909; Mitchell; rather many *Opalina obtrigonoidea orbiculata.*

* No. 13095, 45 mm. long; New Orleans, Louisiana; 1883; R. W. Schufeldt; *Opalina obtrigonoidea orbiculata.*

* No. 37837, 38 mm. long, Dorchester County, Maryland; July, 1907; W. P. Hay; many *Opalina obtrigonoidea orbiculata.*

No. 37835; same label; no Opalinids.

*No. 12005, 42 mm. long; Georgiana, Florida; W. Wittfield; *Opalina obtrigonoidea orbiculata.*

No. 12005, 5 other specimens, 31 to 45 mm. long; same label; no Opalinids.

* No. 3655, 39 mm. long; Pensacola, Florida; Hammond; numerous *Opalina obtrigonoidea orbiculata.*

No. 3655, another specimen; same label; no Opalinids.

No. 3657, 2 specimens, each 50 mm. long; Prairie Mer Rouge, Louisiana; Fairlie; no Opalinids.

No. 13101, New Orleans, Louisiana; R. W. Schufeldt; no Opalinids.

H. dolichopsis (Cope), No. 57717, 120 mm. long; Duke of York Island; 1911; Julius Hurter, sr.; no Opalinids.

*No. 57718, 70 mm. long; Sorong, Dutch Papua; 1906; Julius Hurter, sr.; fine *Protoopalina papuensis.*

* *H. evittata* Miller, No. 32106, 43 mm. long; Easton, Maryland; September 8, 1903; H. L. Clark; many *Opalina terrae-mariae.*

No. 30722, 48 mm. long; Eastern Shore of Maryland; W. P. Hay; no Opalinids (formalin?).

No. 29653, 39 mm. long; Four Mile Run, Virginia; July 4, 1901; G. S. Miller; no Opalinids (formalin?).

No. 27452, 32 mm. long, Lexington County, Virginia; G. S. Miller; no Opalinids, *Balantidium.*

H. ewingii Duméril and Bibron, Australia; examined by Raff; no Opalinids.

* *H. eximia* Baird, No. 11357, 26 mm. long; Guanajuato, Mexico; June 30, 1880; Dugés; many *Opalina virguloidea.*

* No. 9875, 30 mm. long; female with eggs; Guanajuato, Mexico; 1877; A. Dugés; many *Opalina virguloidea.*

* No. 14601, 20 mm. long; Mexico; September 1; A. Dugés; many *Opalina virguloidea.*

No. 14601, 2 other specimens, 23 and 24 mm. long; same label; no Opalinids.

No. 9898, 30 mm. long, Mexico; 1877; A. Dugés; no Opalinids.

No. 9338, 35 mm. long; Santa Fe, New Mexico; June, 1874; H. W. Henshaw; no Opalinids.

No. 25232, 34 mm. long; "near Guatemala"; Van Patten; no Opalinids.

* *H. femoralis* Daudin, No. 5598, 33 mm. long; female with eggs, in copulation; Liberty County, Georgia; Major LeConte; a few *Opalina obtrigonoidea* of ordinary size, no cysts.

* No. 5598, male, 27 mm. long, in copulation; same label; many *Opalina obtrigonoidea*, no cysts.

*No. 5908, 2 specimens, 27 and 32 mm. long; Riceboro, Georgia; Maj. John LeConte; a few *Opalina obtrigonoidea.*

No. 5908, another specimen. 36 mm. long; female with eggs; same label; no Opalinids.

*No. 48783, 33 mm. long; Auburndale, Florida; March, 1912; N. R. Wood; a very few *Opalina obtrigonoidea.*

No. 9698, 34 mm. long; Arlington, Florida; G. Brown Goode; no Opalinids, *H. fleischmanni* Boettger, No. 29933, Juarrialba, Costa Rica; Burgdorf and Schild; no Opalinids (formalin?).

No. 29978, 28 mm. long; San Carlos, Costa Rica; Burgdorf and Schild; no Opalinids (formalin?).

No. 29891, 22 mm. long; Costa Rica; Burgdorf and Schild; no Opalinids.

No. 29928, 19 mm. long; Costa Rica; Burgdorf and Schild; no Opalinids.

No. 19601, 25 mm. long; Rio Frio, Costa Rica; February 29, 1893; C. W. Richmond; no Opalinids.

No. 48727, La Estrella, Costa Rica; June 11; A. Alfaro; no Opalinids (formalin?).

H. lesueurii Duméril and Bibron, several specimens; Australia; examined by Raff; no Opalinids.

**H. nassica* Cope, No. 6226, 37 mm. long; Paraguay; Captain Page; a few *Cepedea dimidiata* [*paraguensis*].

H. peronii (Tschudi), No. 26408, 51 mm. long; female with eggs 1½ mm. in diameter; Australia; A. D. Ogilby; no Opalinids.

Australian specimens examined by Raff, who reports no Opalinids from them.

No. 62746, 45 mm. long; Tamworth, New South Wales, Australia; November 2, 1919; C. M. Hoy; no Opalinids.

No. 62744, 38 mm. long; Wandandian, New South Wales, Australia; August, 1919; C. M. Hoy; no Opalinids.

**H. pickeringii* (Holbrook), numerous living specimens from Raleigh, North Carolina; April, 1915; H. H. and C. S. Brimley; for the most part abundantly infected with *Opalina pickeringii*, many in division and with numerous cysts.

One living specimen; Woods Hole, Massachusetts; August 13, 1919; M. M. Metcalf; no Opalinids.

*No. 15260, 25 mm. long; female with eggs in oviduct; District of Columbia, United States of America; H. W. Henshaw; a few *Opalina obtrigonoidea.*

*No. 5380. 30 mm. long; female with eggs; Selkirk Settlement; R. Kennicott; rather many *Opalina obtrigonoidea.*

*No. 3604, 2 specimens, each 20 mm. long, one a female with eggs; Aux Plains River, Illinois; R. Kennicott; *Opalina obtrigonoidea.*

No. 3604, another specimen; same label; no Opalinids.

**H. pickeringii* × *H. andersoni* (?), living specimen; Oberlin, Ohio; many *Opalina obtrigonoidea.*

**H. pulchella* Duméril and Bibron, No. 5407, 30 mm. long; Paraguay; Captain Page; rather many *Zelleriella hylaxena.*

No. 5407, 23 mm. long, female with eggs; same label; no Opalinids.

Six specimens from an unnamed locality in Uruguay were found by Cordero (1919) to bear no Opalinids.

**H. regilla* Baird and Girard, No. 31905, 40 mm. long; Three Sisters, Oregon; 5.000 altitude; July 17, 1904; V. Bailey; many *Opalina oregonensis.*

*No. 52321, 27 mm. long, Los Angeles County, California; E. J. Brown; rather many *Opalina virguloidea.*

Nos. 52319, 52325, 37 and 21 mm. long; same label; no Opalinids.

*No. 22599, 40 mm. long, Vancouver Island, British Columbia; June 2, 1895; True and Prentiss; rather many *Opalina oregonensis.*

No. 22598, 35 mm. long; same label; no Opalinids.

No. 52382, 31 mm. long; Portland, Oregon; April 19, 1913; O. J. Murie; no Opalinids.

H. rubra Daudin, 40 mm. long; Para, Brazil; "British Museum"; no Opalinids.

Nos. 48859, 48860, 30 and 33 mm. long; Bonito, Brazil; J. C. Branner; no Opalinids.

* *H. septentrionalis* Boulenger, No. 51867, 80 mm. long; female with eggs; Los Hermanos Mountains, Cuba; May 31, 1914; P. Bartsch; many *Opalina* sp. (?).

* No. 27420, 43 mm. long; Pinar del Rio, Cuba; February 21, 1900; Palmer and Riley; rather many *Opalina* sp. (?).

* No. 5096, 3 specimens, 48, 55, and 70 mm. long; Cuba (?); C. Wright; all with *Opalina* sp. (?).

No. 36602, 68 mm. long; Bahama Islands; T. Barbour; no Opalinids, *Nyctotherus.*

* No. 32029, 60 mm. long; New Providence, Bahama Islands; June 19, 1903; United States Fish Commission; many *Zelleriella* [of *Hyla septentrionalis*], also *Opalina* sp. (?).

No. 32030, 32033, 45 and 44 mm. long; same label; no Opalinids, one contained *Balantidium.*

No. 32026, 55 mm. long; female with eggs all laid; Long Island, Bahama Islands; July 16, 1903; Riley; no Opalinids.

H. simplex Boettger, No. 33127, 38 mm. long; Tonkin, China; Kny-Scheerer Co.; no Opalinids.

* *H. venulosa* (Laurenti), No. 27797, 95 mm. long; La Guaira, Venezuela; July 3, 1900; Lyon and Robinson; a few *Zelleriella venezuelae.*

Nos. 35141, 35142, 80 and 85 mm. long; both females with eggs; Guatemala; H. Berendt; no Opalinids.

No. 38265, 63 mm. long; female with eggs mostly laid; found in Tillandsia along with pale colored tadpoles, Cordoba, Mexico; March 21, 1868; Knab; no Opalinids.

Nos. 13975, 13976, 70 and 75 mm. long; Nicaragua; August 28, 1884; J. A. McNeil; no Opalinids.

No. 36377, 75 mm. long; female with a few eggs left in the oviduct; Chicara, Venezuela; June, 1905; G. K. Cherrie; no Opalinids, *Balantidium.*

No. 22545, 77 mm. long; La Guaira, Venezuela; June 22, 1895; W. Robinson; no Opalinids.

* *H. versicolor* LeConte, a half-grown living specimen; Leland, Michigan; July 18, 1917, M. M. Metcalf; very many *Opalina hylaxena.*

* Seven living tadpoles from Woods Hole, Massachusetts; July 21, 1919; M. M. Metcalf; all well infected either with *Opalina hylaxena* form *orbiculata*, or with *O. hylaxena* form *parvinucleata.*

Two adults, Woods Hole, Massachusetts; July 8, 1919; M. M. Metcalf; no Opalinids.

* No. 44550, 45 mm. long; Tate, Georgia; July 4, 1908; Howell; a good many *Opalina hylaxena* form *georgiana.*

* No. 50667, 33 mm. long; Anderson, Indiana; September 23, 1900; W. Knowlton; rather many *Opalina hylaxena* form undetermined.

No. 49726, 33 mm. long; Plummer Island, Maryland; Biological Survey; no Opalinids.

No. 51437, 33 mm. long; Autauquaville, Alabama; May 10, 1914; Peters; no Opalinids.

No. 49570, 35 mm. long; Long Corner, Maryland; July, 1912; S. O. Burdette; no Opalinids.

No. 42630, 50 mm. long; Lake Mackinkuckee, Indiana; September 7, 1906; Evermann and Clark; no Opalinids; a few *Balantidium.*

Nos. 45517, 45518, 45 and 39 mm. long; Mer Rouge, Louisiana; June 2, 1892, Bailey; no Opalinids.

* *H. versicolor chrysoscelis* Cope, No. 3234, 2 specimens, each 40 mm. long; New Braunfels, Texas; Lindheimer; rather many *Opalina* sp. (?).

Nos. 21489, 21490, 45 and 42 mm. long; Hot Springs, Arkansas; June 25, 1894; Brimley; no Opalinids.

No. 36399, 42 mm. long; male; Refugio County, Texas; June, 1904; F. Sherman (?); no Opalinids.

No. 36400, 42 mm. long; Hot Springs, Arkansas; June, 1895; no Opalinids.

Nototrema [*Notodelphys*] *marsupiatum* Duméril and Bibron, No. 12330, 50 mm. long; female with small ovaries and a few large eggs; Paraguay; Paraguay Expedition; no Opalinids (poorly preserved, very soft).

No. 33863, 63 mm. long; Guamote, Ecuador; 8,500 feet altitude; October 10, 1903; S. A. Davis; no Opalinids (formalin?).

* *Phyllomedusa dacnicolor* (Cope), No. 6037, 2 specimens 45 and 33 mm. long; Mexico; Bischoff; many *Opalina helenae phyllomedusae; Balantidium* also was present in the larger individual.

No. 6037, another specimen 55 mm. long; same label; no Opalinids.

No. 14081, 48 mm. long; Presidio, Mexico; April 3, 1885; A. Forer; no Opalinids.

Ph. hypochondrialis (Daudin), No. 48857, 36 mm. long; Bonito, Pernambuco Province; Brazil; J. C. Branner; no Opalinids.

**Ph. lemur* Boulenger No. 29935, 22 mm. long; Jurialba, Costa Rica; Burgdorf and Schild; rather many *Cepedea globosa.*

Family BUFONIDAE.

**Bufo americanus* Holbrook, numerous living infections from Raleigh, North Carolina; April 14, 1912; H. H. and C. S. Brimley, collectors. Many *Opalina obtrigonoidea americana* and its form *rugosa.*

*Several living specimens from Oberlin, Ohio, M. M. Metcalf, collector. Many *Opalina obtrigonoidea americana* and its form *rugosa.*

*Twelve living toads from Raleigh, North Carolina; April, 1915; H. H. and C. S. Brimley, collectors; two infections, one of *Opalina obtrigonoidea americana* and one of its form *rugosa.*

**B. arenarum* Hensel, Cordero (1919) reports a form, which he identifies with *Zelleriella antilliensis*, from Montevideo, Uruguay.

B. asper Gravenhorst, No. 43891, 127 mm. long; Java; May, 1909; Bryant Expedition, cysts, only, of Opalinidae.

* No. 43890, 140 mm. long; Buitensorg, Java; March 31, 1909; cysts, only, of Opalinidae.

*No. 30990, 140 mm. long; Malay Peninsula; June 22, 1892; W. L. Abbott; cysts, only, of Opalinidae.

No. 43310, 127 mm. long; Java; March 16, 1909; Bryant Expedition; no Opalinids.

B. auritus Cope, No. 32327, 45 mm. long; west coast of Costa Rica; no Opalinids.

B. biporcatus Tschudi, No. 7135, 45 mm. long; no Opalinids.

No. 43207, 80 mm. long; Depok, Java; June 23, 1909; Bryant Expedition; no Opalinids.

*No. 29440, 51 mm. long; Sumatra, December, 1901; D. L. Karcher; a few cysts, apparently of some species of Opalinid.

B. borbonicus Tschudi, No. 43206, 57 mm. long; Java; March 3, 1909; Bryant Expedition; no Opalinids.

*_B. boreas_ Baird and Girard. No. 48645, 48646. 41 and 52 mm. long; Head of Moose River; British Columbia; July 13; J. H. Riley; many _Opalina obtrigonoidea maxima_, the smaller specimen held also many _Balantidium_.

*No. 50915, 76 mm. long; Haines, Alaska; August 6, 1913; E. P. Walker; myraids of _Opalina obtrigonoidea maxima_, also _Balantidium_.

* No. 50917, 38 mm. long; same label; _Opalina obtrigonoidea maxima_ of smaller size, also _Balantidium_.

*No. 50914, 82 mm. long; same label; a few cysts only.

No. 50916, 51 mm. long; same label; no Opalinids; a few huge _Balantidium_.

*Nos. 50918, 50919, each 70 mm. long; female with eggs; Boca de Quadra, Alaska; August 30, 1913; E. P. Walker. One of these was uninfected, the other held a tremendous number of _Opalina obtrigonoidea maxima_, the heaviest infection I have ever seen.

No. 48647, 82 mm. long; female with eggs; Henry House, Alberta; September 7; J. H. Riley; no Opalinids.

No. 48636, 61 mm. long; Moose Lake, British Columbia; August 4, 1911; N. Hollister; no Opalinids.

*?No. 48637, 42 mm. long; same label; "a very few stocky Opalinids" is recorded in my notes, but in working over the material I do not again find them.

*No. 48638, 61 mm. long; same label; a very few _Opalina obtrigonoidea maxima_.

No. 48624, 70 mm. long; Prairie Creek, Alberta, Canada; July 3; J. H. Riley; no Opalinids.

*No. 48625, 57 mm. long; Athabasca, Canada; July 4, 1911; J. H. Riley; a single large _Opalina obtrigona maxima_.

*_B. bufo_ (Linnaeus) [=_B. vulgaris_], many observers report from Europe in this toad _Cepedea dimidiata_, and _Opalina ranarum_, and Collin reports _Opalina cincta_ from Viarmes, France.

*_B. bufo asiaticus_ (Steindachner), No. 21214, 105 mm. long; female with eggs; Seoul, Korea; August 8, 1883; P. L. Jouy; numerous _Protoopalina axonucleata_.

*No. 21215, 51 mm. long; same label; a few _Protoopalina axonucleata_.

*No. 52355, 64 mm. long, also No. 52353; southern Manchuria; A. de C. Sowerby; _Opalina_ [_bufoxena_].

*No. 52356, 51 mm. long; same label; a few _Protoopalina axonucleata_.

*No. 46617, 101 mm. long; Shanghai, China; D. C. Jansen; a few _Opalina_.

*No. 49642, 72 mm. long; 20 miles southeast of Tai Yuan Fu, Shensi, China; 2,300 feet altitude; September 9, 1911; A. de C. Sowerby, many _Opalina_ of a species not determined. The parasites in this very abundant infection are so crowded together and distorted that one can not properly study them. They seem to be _Opalina_ [_bufoxena_].

*_B. calamita_ Laurenti, Brumpt (1915) reports _Protoopalina intestinalis_ from an unnamed locality.

*Collin (1913) reports cysts of some Opalinid from this host at Montpellier, France.

*?_B. canaliferus_ Cope, No. 10022, 42 mm. long; female with eggs 1 mm. in diameter, Tehuantepec, Mexico, F. Sumichrast, 2 individuals, seemingly of some species of _Cepedea_, were seen, but were lost.

B. coccifer Cope, No. 25233; "near Guatemala"; Van Patten; no Opalinids.

No. 51775, Tuchitan, Mexico; F. Sumichrast; no Opalinids.

**B. cognatus* Say, No. 37969, 84 mm. long; Albuquerque, New Mexico; June 1, 1907; very many *Opalina discophrya.*

*No. 35629, 63 mm. long; Phoenix, Arizona; M. C. Dickerson; many *Zelleriella hirsuta* and some *Opalina discophrya.*

*No. 37138, 57 mm. long; Fort Mohave, Arizona; March 19, 1904; C. W. Adams; *Zelleriella hirsuta.*

No. 19416, 76 mm. long; Texas-Mexico boundary; E. A. Mearns; no Opalinids.

No. 21070, 82 mm. long; Arizona; July 1, 1913; E. A. Mearns; no Opalinids.

**B. compactilis* Wiegmann, No. 26460, 71 mm. long; Texas; very many *Opalina gigantea.*

No. 26459, same label, female with eggs; same label; no Opalinids.

*No. 4964, 2 specimens, each 76 mm. long; Pecos River, Texas; Captain Pope; one was uninfected, the other bore many *Opalina spiralis.*

*No. 38060, 71 mm. long; female with eggs 1 mm. in diameter; Prescott, Arizona; July 6, 1907; J. Hurter; *Balantidium* and myriads of *Opalina spiralis.*

No. 48606, Victoria, Texas; no Opalinids.

No. 20877, 89 mm. long; Fort Clark, Texas; E. A Mearns; no Opalinids.

No. 38356, 82 mm. long; female with eggs 1 mm. in diameter; San Antonio, Texas; May 24, 1908; J. Hurter; no Opalinids, a few *Nyctotherus.*

**B. coniferus* Cope, No. 29976, 39 mm. long; Costa Rica; Burgdorf and Schild; a few *Zelleriela opisthocarya.*

*No. 20703, Nicaragua; October 24, 1892; C. W. Richmond; 2 individuals of *Zelleriella opisthocarya.*

*No. 14194, 51 mm. long; Nicaragua; August 7, 1885; J. F. Bransford; 1 individual of *Zelleriella opisthocarya.*

No. 14195, same label; no Opalinidae, 1 individual of *Balantidium.*

No. 14188, 2 specimens, each 64 mm. long; same label; no Opalinids.

Nos. 14197, 14198, 51 and 38 mm. long, Nicaragua; August 28, 1892; C. W. Richmond; no Opalinids.

**B. copei* Yarrow and Henshaw, No. 5388, 5 specimens, 38 to 51 mm. long; East Hudson Bay; C. Drexler; all but one infected with *Opalina discophrya.*

* No. 5372, 2 specimens, 45 and 64 mm. long; "South of High Land, Ontario"; C. Drexler; the larger had a very few *Opalina discophrya.*

* No. 5376, 20 mm. long; Selkirk Settlement; Donald Gunn; a few *Opalina discophrya* and a few very interesting, elongated, slender *Balantidium.*

No. 5376, 57 mm. long, same label; no Opalinids.

* No. 5367, 62 mm. long; Moose River, "British America"; C. Drexler; 2 individuals of a flat species of *Opalina* different from *O. discophrya* were seen but not studied.

No. 5367, another specimen, 62 mm. long; same label; no Opalinids.

B. cruentatus Tschudi, No. 43208, 51 mm. long; Java; 5,500 feet altitude; August, 1909; Bryant expedition; no Opalinids, many *Balantidium.*

* *B. debilis* Girard, No. 19418, 39 mm. long; Texas-Mexico boundary; E. A. Mearns; a very few cysts of some species of Opalinidae.

Nos. 19422, 19425, 32 and 30 mm. long; same label; no Opalinids.

No. 2623, 39 mm. long; Chihuahua, Mexico; J. H. Clark; no Opalinids.

No. 2624, 45 mm. long; female with eggs, Delaware Creek, western Texas, no Opalinids.

No. 22389, 45 mm. long, female with eggs, San Antonio, Texas, H. H. and C. S. Brimley, no Opalinids.

No. 2619, 25 mm. long, Brazos River, Texas, Schumard, no Opalinids.

No. 25153, 22 mm. long, Mazatlan, Mexico, April, 1868; no Opalinids.

No. 19426, Texas-Mexico boundary, E. A. Mearns, no Opalinids.

B. d'orbignyi Duméril and Bibron, No. 22750, La Plata, South America, no Opalinids.

Cordero (1919) reports *Zelleriella antilliensis*, or a similar form, from Uruguay.

* *B. formosus* Boulenger, No. 54324, 134 mm. long; Mount Fujiyama, Japan; August, 1898; A. Owston; a very few *Cepedea fujiensis.*

Nos. 34323, 34325 to 34328, 102 to 139 mm. long; same label; no Opalinids.

No. 11348, 102 mm. long; Japan; 1878; E. S. Morse; no Opalinids.

Nos. 51997, 51998, 114 and 95 mm. long; both females with eggs, Karizawa, Japan; August, 1914; F. Baker; no Opalinids.

* *B. fowleri* Putnam, 5 living individuals from Wood's Hole, Massachusetts, July, 1919, M. M. Metcalf, one individual infected abundantly with *Opalina obtrigonoidea.*

Nos. 35927, 35928, 70 and 61 mm. long; both females with eggs, Elizabeth Islands, Massachusetts; M. C. Dickerson; no Opalinids.

No. 42269, 64 mm. long; Lanham, Maryland; July, 1910, W. R. Maxon, no Opalinids, *Balantidium.*

* No. 52471, 36 mm. long; Mount Monadnock, New Hampshire, August, 1915, M. J. Rathbun, *Opalina obtrigonoidea* form *plicata.*

No. 52472, 20 mm. long, same label, no Opalinids.

* *B. gargarizans* Cantor, No. 46490, Hong Kong, China, 1883, P. L. Jouy, *Cepedea buergeri sinensis.*

No. 46489, same label, no Opalinids.

* *B. haematiticus* Cope, No. 14181, 9 specimens, 25 to 51 mm. long, Nicaragua, August 7, 1885, J. F. Bransford; one was uninfected; two contained *Balantidium* only; three had *Cepedea dolichosma;* one had this species and also *Opalina* sp. (?); three had *Zelleriella bufoxena.*

* No. 29902, 44 mm. long, Jucurique, Costa Rica, Burgdorf and Schild, 2 specimens only of *Cepedea dolichosoma.*

No. 32557, 76 mm. long; female with eggs 1 mm. in diameter, Costa Rica; Gabb; no Opalinids (poorly preserved, very soft).

* Nos. 38723 to 38725, 38729, each 35 mm. long; Punta de Pena, Panama; July 24, 1908; R. E. B. McKenney; 2 uninfected, 1 showed a single *Balantidium*, 1 showed a single *Zelleriella bufoxena.*

* *B. halophilus* Baird and Girard, in numerous living specimens from San Francisco, California, the author found *Opalina obtrigonoidea maxima.* Nearly all the toads examined were infected.

* *B. intermedius* Guenther, Nos. 26161, 26162, 45 and 22 mm. long; Guanajuato, Mexico; A. Dugès; each showed a good many *Zelleriella intermedia.*

No. 26160, 89 mm. long, same label; no Opalinids.

Nos. 30223 to 30225, 41 to 95 mm. long; Orizaba, Mexico; F. Sumichrast; no Opalinids. One of the two larger toads was a female with immature eggs.

* *B. jerboa* Boulenger, No. 33880, 35 mm. long; western Borneo; F. Werner; a good many *Cepedea borneonensis.*

* *B. latifrons* Boulenger, No. 48855, 76 mm. long; The Cameroons, western Africa; T. Barbour; very many *Cepedea magna*, also many *Nyctotherus.*

No. 48854, same label; very numerous *Balantidium*, no Opalinids.

* B. *lemur* Cope, Nos. 27148 to 27150, 73 to 76 mm. long; Porto Rico; April 4, 1900; L. Stejneger; 1 uninfected, 2 abundantly infected with *Zelleriella microcarya.*

* *B. lentiginosus* Shaw, Nos. 48778 to 48780, 64 to 70 mm. long; Auburndale, Florida; March, 1912; N. R. Wood; *Cepedea obovoidea* and *Opalina triangulata.*

* *B. marinus* (Linnaeus), in about 12 living individuals from Jamacia. West Indies, and from Bermuda, the author has found *Zelleriella antillensis.* Most of the toads examined were infected.

* *B. mauritanicus* Schlegel, Brumpt (1915) reports *Protoopalina intestinalis*, locality not mentioned.

* *B. melanosticus* Schneider, No. 37511; Hong Kong, China; Dale and Jouy; a few *Cepedea formosae.*

* Nos. 36498, 36499, 89 and 60 mm. long; Formosa; September, 1896; T. Tada. The smaller specimen bore a few *Cepedea formosae* and the other specimen bore a few Opalinid cysts.

* Bezzenberger reports *Zelleriella macronucleata* in toads of this species from "Asia." We have hesitated to accept this report without confirmation. (See p. 327.)

* No. 38206, 89 mm. long; Formosa; June 5, 1907; H. Sauter; many *Protoopalina formosae.*

Nos. 38207, 38208, same label, no Opalinids.

Nos. 43893 to 43898, 54 to 64 mm. long; Java; Bryant Expedition; no Opalinids.

Nos. 43879 to 43889, 71 to 114 mm. long; Buitensorg, Java; Bryant Expedition; no Opalinids.

Nos. 43893, 43900, 43901; 39 to 51 mm. long, Java; Bryant Expedition; no Opalinids.

Nos. 38203 to 38205, 75 to 127 mm. long; Formosa; June 5, 1907; H. Sauter; no Opalinids, a few *Discophrya.*

No. 29439; Sumatra; Deli; D. L. Karcher; no Opalinids.

No. 43892, 43 mm. long; Tjibodas, Mount Gede, Java; April 21, 1908; Bryant Expedition; no Opalinids.

No. 43902, 83 mm. long; Depok, Java; July 18, 1909; Bryant Expedition; no Opalinids.

* No. 43944, 24 mm. long, Buitenzorg, Java; March 20, 1909; Bryant Expedition; very many *Cepedea pulchra javensis.*

No. 43904, 54 mm. long. Depok; Java; July 18, 1909; Bryant Expedition; no Opalinids.

Nos. 53519, 53520, 70 mm. and 50 mm. long, Klong Yai, southeastern Siam; December, 1914, January, 1915; C. Borden Kloss; no Opalinids.

B. molitor Tschudi, No. 12273, 3 specimens, 89 to 101 mm. long; Guayaquil, Ecuador; no Opalinids.

* *B. monxiae* Cope, No. 12290, 22 mm. long; Yucatan; *Zelleriella opisthocarya* [of *Bufo monxiae*].

* No. 11358, 29 mm. long, Guanajuato, Mexico; January 30, 1880; A. Dugés; a very few *Zelleriella opisthocarya* [of *Bufo monxiae*].

No. 11359, 22 mm. long; same label; no Opalinids.

* *B. peltocephalus* Tschudi, No. 51864, 125 mm. long; Cuba, June 3, 1914; J. B. Henderson and P. Bartsch, *Protoopalina bufonis* and *Zelleriella* sp.?.

* No. 51865, same label. a single *Protoopalina bufonis* found.

* No. 27330, 152 mm. long; female with eggs; Pinar del Rio, Cuba; March 18, 1900; W. Palmer and J. H. Riley; a very few *Protoopalina bufonis.*

No. 29838, 29840, 113 and 108 mm. long; Cuba; 1902; William Palmer; no Opalinids.

No. 29844, 146 mm. long; El Cobre, Cuba; William Palmer; no Opalinids.

No. 28422, 152 mm. long; Santiago, Cuba; J. H. Hysell; no Opalinids.

* *B. punctatus* Baird and Girard, 12661, 4 specimens, La Paz, California; 1882; L. Belding; all infected with *Zelleriella* [of *Bufo punctatus*].

* No. 26159, 41 mm. long; Guanajuato, Mexico; A. Dugés; a good many *Zelleriella* [of *Bufo punctatus*], also *Opalina obtrigonoidea*.

No. 26158, same label; no Opalinids.

* No. 18769, 57 mm. long, female with eggs, Furnace Creek, Death Valley, California, March 21, 1891, many *Zelleriella* [of *Bufo punctatus*].

Nos. 18759, 18772, 41 and 45 mm. long, same label, *Zelleriella* [*of Bufo punctatus*].

Nos. 18756, 18761, each 45 mm. long; same label; no Opalinids.

Nos. 39499, 39500, each 57 mm. long; California (?); no Opalinids.

Nos. 18784, 18785, 45 and 22 mm. long; Cottonwood Canyon, Panamint Mountains, California; 3,800 feet altitude; May 29, 1891; Nelson; no Opalinids.

* *B. raddei* Strauch, Nos. 53387 to 53389, 57, 70 and 57 mm. long; Hei Sui, northeastern Chili, China; A. de Sowerby; very many *Opalina raddei*.

* No. 39370, 62 mm. long, Kansu, China, July 18, 1909, A. de C. Sowerby, a few *Opalina raddei*.

* No. 53372, 76 mm. long, Hei Sui, northeastern Chili, China, A. L. Hall, *Opalina raddei*.

* *B. regularis* Reuss, No. 16064, 63 mm. long; Gold Coast, western Africa; December 29, 1889; W. H. Brown; very many *Protoopalina regularis*.

No. 16063, 114 mm. long, same label, no Opalinids.

* Nos. 16041, 16043, 89 and 108 mm. long; West Africa; December 10, 1889; W. H. Brown; *Protoopalina regularis*.

No. 16042, 108 mm. long; same label; no Opalinids.

* No. 20121, 38 mm. long, Tana River, British East Africa, November, 1892, W. A. Chanler, a few *Protoopalina regularis*.

Nos. 20116 to 20120, 32 to 79 mm. long; same label; no Opalinids.

No. 20122, 38 mm. long; same label; no Opalinids.

No. 20107, 76. mm. long; same label; no Opalinids.

* Stevenson (1911) reports from this toad from Khartoum, Sudan, a species I have named *Protoopalina stevensoni*.

B. simis Schmidt, No. 38946, 28 mm. long; Tobago Island, Bay of Panama; T. Barbour; no Opalinids.

* *B. smithi* Stejneger, No. 31947, 63 mm. long; Kochi, Japan; May, 1903; H. M. Smith; numerous *Opalina ranarum smithi*.

* No. 31946, same label, numerous Opalinid cysts.

Five other specimens from the same jar, 64 to 152 mm. long, no Opalinids.

**B. spinulosus* Weigmann, No. 38575, 63 mm. long, Lake Titicaca, Peru, July 31, 1908, R. E. Coker, very many *Zelleriella* [of *Bufo spinulosus*], also cysts.

* No. 38938, 76 mm. long, La Paz, Brazil; T. Barbour; a few *Zelleriella* [of *Bufo spinulosus*].

* *B. sternosignatus* Keferstein, No. 30434 to 30436, 63 to 76 mm. long; Tehuantepec, Mexico; F. Sumichrast; each gave a few *Zelleriella* [of *Bufo sternostignotus*].

No. 30431 to 30433, 57 to 70 mm. long, same label; no Opalinids.

No. 10014, 2 specimens, male and female, each 63 mm. long; Tehuantepec, Mexico; February, 1876; F. Sumichrast; no Opalinids.

**B. typhonius* (Linnaeus), No. 25181, 25176, 16 and 32 mm. long, Bahia Solado, Panama, J. F. Bransford, each infected with *Zelleriella opisthocarya* [of *Bufo typhonius*], and *Opalina panamanensis.*

No. 25177 to 25179. 16 to 31 mm. long. same label, no Opalinids.

**B. valliceps* Weigmann, No. 52279, 82 mm. long, Brownsville, Texas, January 1915, R. D. Camp, *Zelleriella intermedia cuneata.*

*No. 52297, 57 mm. long; Brownsville, Texas; March 31, 1915; R .D. Camp; many *Zelleriella intermedia cuneata.*

*No. 25138, Guatemala, Berendt, a few *Zelleriella intermedia cuneata.*

*No. 24849, 63 mm. long. Guatemala, H. Hogue, a few *Zelleriella intermedia cuneata.*

Nos. 24847. 24848, same label, no Opalinids.

No. 53179, 76 mm. long; New Orleans, Louisiana; May 1915, Southern Biological Supply Co.; *Balantidium*, no Opalinids.

B. variegatus (Guenther), Nos. 15123, 15124, 35 mm. long, both female with eggs, Mayne Harbor, Patagonia, February 5, 1888. no Opalinids, one host contained *Balantidium.*

**B. viridis* Laurenti. Of 4 specimens from Naples, Italy, Metcalf (1909) reported one bearing *Protoopalina caudata* and one with *Opalina ranarum* and one with *Cepedea dimidiata.* Preserved material collected at the same place has since yielded *Cepedea dimidiata* from each of two other specimens.

* *B. woodhousi* Girard, Nos. 52144 to 52146, 19 to 24 mm. long; Arizona; August, 1914; J. S. Ligon; each showed a few *Opalina woodhousi.*

*No. 36364, 63 mm. long; Provo City, Utah; June 26, 1905; *Zelleriella* [of *Bufo woodhousi*] and *Opalina woodhousi.*

*No. 57133, 63 mm. long; Arizona; July 14, 1914; many *Opalina woodhousi.*

No. 39686, 63 mm. long; Provo City, Utah; August, 1899; no Opalinids.

No. 21442. 82 mm. long; Redlands, South Dakota; August 28, 1894; no Opalinids.

No. 38051, 84 mm. long, male, Albuquerque, New Mexico, July 9, 1907, *Balantidium*, no Opalinids.

No. 37968, 95 mm. long, female with eggs, Albuquerque, New Mexico, July 9, 1907, no Opalinids.

No. 40206. 29 mm. long, Rangeley, Colorado, September 12, 1906, M. Carey, no Opalinids.

No. 40207, 57 mm. long, Rifle, Colorado, August 15, 1907. M. Carey. very many *Balantidium*, no Opalinids.

No. 52384, 95 mm. long, Portland, Oregon, April 30, 1913, O. J. Mur'e, no Opalinids.

No. 39852, 76 mm. long, female with eggs, Keosho, Missouri. April 3, T. M. Williams, no Opalinids.

No. 39853, 70 mm. long, same label, no Opalinids.

No. 35630, 139 mm. long, Phoenix, Arizona, M. C. Dickerson, no Opalinids.

No. 9471, 139 mm. long, Arizona, no Opalinids.

No. 2535, 140 mm. long. Yellowstone River, July 14. 1856, Hayden, no Opalinids.

No. 4195, 127 mm. long, female with eggs, Platte Valley. Drexler, no Opalinids.

**Engystomops pustulosus* (Cope), No. 30254, 26 mm. long; Mexico (?); F. Sumichrast; many *Zelleriella* [*engystomopsis*].

*Nos. 10025, 10026, each 26 mm. long, 1 female with white eggs 1½ mm. in diameter; Tehuantepec, Mexico; F. Sumichrast; many *Zeleriella* [*engystomopsis*].

No. 10028, same label; no Opalinids.

**E. stentor* (Espada), Nos. 51957 to 51959, 32 to 35 mm. long; Taboga Island, Panama; June 12, 1914; J. Zatek; *Zelleriella* [*engystomopsis*].

Notaden bennetti Guenther, No. 32676, 70 mm. long, Rock Bay, Western Australia; Novara; *Balatidium*, no Opalinids.

Rhinophrynus dorsalis Duméril and Bibron, No. 6622, 2 specimens, each 41 mm. long; Tobasco, Mexico; Laszbo; no Opalinids.

Nos. 25213, 25214, each 51 mm. long, one a female with eggs 1½ mm. in diameter; Vera Cruz, Mexico; L. Linucum; no Opalinids.

No. 25134, Peten, Guatemala, Dr. Berendt, no Opalinids.

Family LEPTODACTYLIDAE.

Ceratophrys ornata (Bell), No. 12167, 57 mm. long, Buenos Ayres, Argentina, no Opalinids.

No. 22753, 90 mm. long, La Plata, Argentina, "Museo de la Plata," no Opalinids.

Crinia laevis froggatti (Fletcher), 2 specimens, Australia, examined by Raff, no Opalinids.

**C. signifera* Girard, Narbethong, near Healesville, Australia, O. B. Davies, *Protoopalina tenuis* reported by Raff.

*Mintone, Australia, *Protoopalina tenuis*, reported by Raff.

No. 26411, 19 mm. long, female with eggs ⅝ mm. in diameter, Australia, J. D. Ogilby, a few *Protoopalina tenuis.*

*No. 26412, 16 mm. long; Australia; J. D. Ogilby; a few *Protoopalina tenuis.*

No. 62742, 22 mm. long, female full of eggs; Wandandian, New South Wales, Australia; August 1, 1919; C. M. Hoy; no Opalinids.

Eleutherodactylus antillensis (Reinhardt and Luetken), No. 27078, Vieguez Island; March 22, 1900; L. Stejneger; no Opalinids.

Another specimen, Vieguez Island, Richmond; no Opalinids.

E. augusti Dugés, No. 13633, 45 mm. long, Helotes, Texas, November 30, 1883, G. W. Marnock, no Opalinids.

No. 48087, 70 mm. long; Helotes, Texas; 1911; G. W. Marnock; no Opalinids (formalin?).

E. auriculatus (Cope), No. 25606, 36 mm. long, Adjuntas, Porto Rico, July 29, 1889, A. D. Baker, no Opalinids (formalin?).

No. 25545, 31 mm. long; Lares, Porto Rico; January 21, 1899; A. D. Baker; no Opalinids (formalin?).

No. 25744, 30 mm. long; Caguas, Porto Rico, January 9, 1899, United States Bureau of Fisheries; no Opalinids (formalin?).

Nos. 25751, 25752, each 24 mm. long, Aibonita, Porto Rico, February 3, 1899; no Opalinids (formalin?).

Nos. 25620, 25621, each 30 mm. long, Aguas Buenas, Porto Rico, January 12, 1899, A. D. Baker; no Opalinids.

No. 25754, 16 mm. long, Aibonita, Porto Rico, February 3, 1899, United States Bureau of Fisheries; no Opalinids.

No. 25774, 40 mm. long, Humacao, Porto Rico, January 5, 1899, McCormick; no Opalinids.

Nos. 25625, 25626, each 28 mm. long, Aguas Buenas, Porto Rico, Baker; no Opalinids.

**E. binghami* Stejneger, No. 59558, 32 mm. long, Urubamba, Peru, 9,500 feet altitude, "under wet stone," July 12, Yale Peruvian Expedition, *Zelleriella binghami.*

* No. 48560, 22 mm. long; Cuzco, Peru; 11,000 feet altitude, July 9; Yale Peruvian Expedition; a few *Zelleriella binghami.*

E. brocchi (Boulenger), Nos. 48281, 48282, 16 and 21 mm. long; La Mica, Costa Rica; A. Alfaro; no Opalinids.

E. diastema (Cope), No. 48728, 13 mm. long; La Estrella, Costa Rica; A. Alfaro; no Opalinids.

E. fleischmanni Boettger, No. 20698, 37 mm. long; Graytown, Nicaragua; October 28, 1892; Richmond; no Opalinids.

Nos. 28716, 28717, 40 and 27 mm. long; Punta de Pana, Panama; July 24, 1908; McKenney; no Opalinids.

* *E. footei*, Stejneger, No. 49563, 19 mm. long; Cuzco, Peru, 11,500 feet altitude; Yale Peruvian Expedition; *Zelleriella cusconis.*

No. 49561, 19 mm. long; Tincochaca, Peru; 7,000 feet altitude, August 8; Yale Peruvian Expedition; no Opalinids.

* *E. leptopus* (Bell), No. 15125, 19 mm. long; Mayne Harbor, Patagonia; February 5, 1887; *Protoopalina diplocarya.*

E. martinicensis (Tschudi), No. 10121, 32 mm. long; Tobago Island, West Indies; 1878; F. A. Ober; no Opalinids.

No. 11253, 2 specimens, each 35 mm. long; Zacatecas, Central Mexico; May, 1880; L. Gueade; no Opalinids (very soft).

E. monensis (Meezwarth), No. 29389, 33 mm. long; Mona Island, Porto Rico; August 9, 1901; B. S. Bowdish; no Opalinids (very soft).

E. polyptichus (Cope), Nos. 29899, 29962, 29975, 17, 17, and 20 mm. long; San Carlos, Costa Rica; Burgdorf and Schild; no Opalinids (formalin ?).

E. recordii (Duméril and Bibron), No. 8310, 22 mm. long; Chihuahua, Mexico; Potts; no Opalinids.

No. 30955, Lemon City, Florida; E. J. Brown; no Opalinids.

E. richmondi Stejneger, Porto Rico; many formalin specimens yielding no Opalinids.

E. rugosus (Peters), Nos. 29894, 29896, 22 and 20 mm. long; Costa Rica; Burgdorf and Schild; no Opalinids (formalin ?).

* *Leptodactylus albilabris* (Guenther), No. 10029, 4 specimens 25 to 30 mm. long; Tehuantepec, Mexico; Sumichrast; one uninfected, one scantily, and two abundantly infected with *Zelleriella leptodactyli.*

* No. 27749, 35 mm. long; Mayaguez, Porto Rico; September, 1900; Bowdish; many *Zelleriella leptodactyli.*

No. 27750, same label; no Opalinids.

* No. 27053, 37 mm. long; Luguillo, Porto Rico; March 4, 1900; Richmond; numerous *Zelleriella leptodactyli.*

No. 26821, 35 mm. long; Mameyez, Porto Rico; March 3, 1900; L. Stejneger; no Opalinids (formalin ?).

Nos. 27103, 27109, 45 and 35 mm. long; Vieguez Island, Porto Rico; March 3, 1900; L. Stejneger; no Opalinids (formalin ?).

No. 27765, 32 mm. long; Tehuantepec, Mexico; Sumichrast; no Opalinids.

* *L. caliginosus* Girard, No. 30116, 42 mm. long; female with immature eggs; Tehuantepec, Mexico; numerous *Zelleriella leptodactyli.*

No. 30117, same label; no Opalinids.

No. 14080, 2 specimens, 25 and 30 mm. long; Presidio, Mexico; Porrer; no Opalinids.

* *L. gracilis* (Duméril and Bibron), Nos. 10018, 10019, 34 and 37 mm. long; Tehuantepec, Mexico; Sumichrast; many *Zelleriella leptodactyli.*

L. insularum Barbour, No. 52404, 90 mm. long; Taboga Island, Bay of Panama; Burgdorf and Schild; no Opalinids.

* *L. microtis* (Cope), Nos. 38102, 38103, 38109, 37, 27, and 23 mm. long; Lake Amatitlan, Guatemala; February, 1906; S. E. Meek; *Zelleriella leptodactyli*.

* No. 38107, 35 mm. long; same label; *Zelleriella leptodactyli, Nyctotherus*, and *Balantidium*.

* *L. ocellatus* (Linnaeus), No. 22749, 76 mm. long; La Plata, South America; many *Zelleriella brasiliensis*.

No. 22748, 70 mm. long; same label; no Opalinids.

* Cordero (1919) reports a form which he identifies with *Zelleriella antilliensis*, from La Plata.

L. pentadactylus (Laurenti), No. 38714, 144 mm. long; Panama; R. E. B. McKenney; no Opalinids. (This animal was abnormal, great adhesions all along the upper rectum.)

No. 16618, 38 mm. long; Trinidad, West Indies; Gill; no Opalinids.

* *L. prognathus* Boulenger. Cordero (1919) reports a form which he identifies with *Zelleriella antilliensis*, from Tacuarembo, Uruguay.

L. rubido (Cope), No. 49553, 44 mm. long; San Miguel; 6,000 feet altitude; Yale Peruvian Expedition; no Opalinids.

* *L. typhonius* (Daudin), No. 36370, female with huge fat-bodies, 45 mm. long; Chicara, Venezuela; "Brooklyn Institute," *Zelleriella magna*.

No. 36369, 45 mm. long, female with eggs, same label; no Opalinids.

* *Limnodynastes dorsalis* (Gray), from Australian specimens Raff reports *Zelleriella binucleata*, *Protoopalina* "*intestinalis*," *P. dorsalis* and *P. acuta*, also "abnormal forms (see fig. 101, p. 136); also Cleland and Johnston (1919) report "*Opalina* sp." [doubtless a *Protoopalina*] from Queensland, Australia.

L. peronii (Duméril and Bibron), No. 26409, 59 mm. long; Sydney, Austrania; J. D. Ogilby; no Opalinids.

* No. 62748, 35 mm. long; Wandandian, New South Wales; Australia, August 1, 1919; *Protoopalina peronii*.

Nos. 62740, 62747, 46 mm. and 36½ mm. long; both female with eggs; same label; no Opalinids.

* Cleland and Johnston (1910) report "*Opalina* sp." [doubtless a *Protoopalina*] from Queensland, Australia.

L. tasmaniensis (Guenther), No. 26410, 39 mm. long; Australia; J. D. Ogilby; no Opalinids.

* One specimen, Tasmania, *Zelleriella binucleata*, reported by Raff.

* *Limnomedusa macroglossa* (Duméril and Bibron) from an unnamed locality in Uruguay. Cordero (1919) reports *Zelleriella antilliensis* or a closely related binucleated species.

Lithodytes rhodopsis Cope, No. 10020; Tehuantepec, Mexico; Sumichrast; no Opalinids.

Nos. 24815, 24816, 24 and 34 mm. long; no Opalinids.

Nos. 30325, 30326, 39 and 32 mm. long; Tehuantepec, Mexico; F. Sumichrast; no Opalinids (poorly preserved).

* *Paludicola bibronii* (Tschudi), Nos. 38909, 38910, 38915, 38916, 38918, 38920, 38925, 38930, 8 specimens; Tolcahuano, Chile; Barbour, all infected, some abundantly so, with *Zelleriella paludicolae;* one specimen had also *Balantidium* and *Nyctotherus*.

* Cordero (1919) reports *Zelleriella antilliensis* or a closely related binucleated species from Uruguay. It was probably *Z. paludicolae*.

* *P. brachyops* (Cope) No. 26371, 39 mm. long; Chicara, Venezuela; June 6, 1901; G. K. Cherrie; many *Zelleriella paludicolae.*

* No. 22540, Margarita Island, Venezuela; July 3. 1895; Robinson; numerous *Zelleriella paludicolae.*

* No. 36372, 39 mm. long, "South America;" Brooklyn Institute; a single *Zelleriella paludicolae* found.

* No. 36374, 45 mm. long, female with eggs; same label; a few *Zelleriella paludicolae.*

No. 36375, same label; no Opalinids.

* *P. bufonina* (Bell), Nos. 36875, 36876, 36879 to 36881, 36883, 36884, 7 specimens 25 to 51 mm. long; Straits of Magellan, Patagonia; 1898; Hatcher; one, a female with eggs, infected only with Nematodes, the others all infected with *Zelleriella patagoniensis.*

P. trinitatis Boulenger, No. 15493, 2 specimens, 27 and 29 mm. long, Trinidad, West Indies; J. W. Hart; no Opalinids.

No. 27809, 21 mm. long, San Julien, Venezuela, August 8, 1910; Robinson, *Balantidium;* no Opalinids.

* *Pseudis mantidactyla* (Cope). from an unnamed locality in Uruguay, Cordero (1919) reports *Zelleriella antilliensis* or a closely related binucleated species.

Family DENDROPHRYNISCIDAE.

Pseudophryne semimarmoratus, Australia, 1 specimen, examined by Raff; no Opalinids.

Syrrophus campi Stejneger, No. 52291, 21 mm. long, Brownsville, Texas, March 3, 1915; R. D. Camp; no Opalinids (formalin ?).

S. marnocki Cope, No. 13635, Helotes, Texas, November 30, 1883, G. W. Marnock; no Opalinids.

* *Telmatobius jelskii* (Peters), Nos. 38118, 38119, each 51 mm. long, Ecuador, Barbour, *Zelleriella telmatobii.*

* No. 38577, 32 mm. long, Blanca Island, Peru, July 13, 1908, R. E. Croker, very many *Zelleriella telmatobii.*

* No. 33865, 45 mm. long; Guamote, Ecuador; 10,000 feet altitude; October 10, 1903; S. Austin Davis; a few *Zelleriella telmatobii.*

* No. 33864, 51 mm. long; same label; many *Protoopalina longinucleata.*

* *Uperoleia marmorata* Gray, one specimen; Narbethong, Australia; O. B. Davies; *Protoopalina* "*intestinalis*" reported by Raff, also *Protoopalina tenuis.*

Family GASTROPHRYNIDAE.

Atelopus gracilis Barbour, 52401, 39 mm. long; Gorgona Island, Colombia, South America; W. W. Brown and J. E. Thayer; no Opalinids.

* *A. stelzneri* Weyenbergh, Nos. 28481, 28521, each 26 mm. long; Sapucay, Patagonia; W. T. Foster; many *Zelleriella atelopyxena* [*stelzneri*].

Nos. 28464, 28488, 28502, 28517, 28519, 5 specimens; same label; no Opalinids, one had *Nyctotherus.*

Nos. 40053. 40055, 25 and 27 mm. long; female with eggs 1 mm. in diameter; Moldonado, Uruguay; F. W. Golding; no Opalinids.

No. 40061, 26 mm. long; male; same label; no Opalinids.

No. 38533, 2 specimens, 26 mm. long; no Opalinids.

No. 48608, 22 mm. long; female with eggs; Moldonado, Uruguay; F. Telippone; no Opalinids.

* *A. varius* Stannius, Nos. 30643, 30645, 45 and 32 mm. long; Pico Bianco, Costa Rica; W. M. Gabb; scant infections of *Zelleriella atelopyxena.*

* No. 30633, 39 mm. long; same label; a few *Zelleriella* [*atelopodos*].

Nos. 30644, 30650. 44 mm. long; same label; no Opalinids.

Nos. 29865, 29867, 29870, 29871, 29876, 26 to 47 mm. long; Costa Rica; Burgdorf and Schild; no Opalinids.

Nos. 30569 to 30573, 32 to 45 mm. long; Aguacate Mountains, Costa Rica; W. M. Gabb; no Opalinids (poorly preserved, soft).

Breviceps mossambicus Peters, No. 57875, 31 mm. long; Mozambique, Africa; February, 1913; Julius Hurter, sr.

* *Gastrophryne carolinensis* (Holbrook), No. 3707, 29 mm. long; Columbia, Georgia; Dr. Gesner; a few *Opalina obtrigonoidea.*

* No. 48894, 28 mm. long; Chilesburg, Virginia; July 22, 1910; F. P. Drowne; many *Opalina obtrigonoidea.*

Nos. 48893, 48895, same label; no Opalinids.

Nos. 53187, 53188, female and male taken in copulation, 26 and 20 mm. long; New Orleans, Louisiana; May, 1915; Southern Biological Supply Co.; no Opalinids

No. 26026, 30 mm. long; Sea Glen, Mississippi; April 18, 1887; Evermann; no Opalinids.

No. 50880, 30 mm. long; female with eggs; Edenton, North Carolina; June, 1913; B. Schwartz; no Opalinids.

No. 17533; St. Louis, Missouri; 1891; J. Hurter; no Opalinids.

No. 20540, 29 mm. long; Raleigh, North Carolina; August 2, 1893; H. H. and C. S. Brimley; no Opalinids.

* *G. pictiventris* (Cope), No. 19903, 39 mm. long; Escondillo River, Nicaragua; July 2, 1902; C. W. Richmond; cysts of some species of Opalinid.

* *G. texensis* (Girard), No. 52296, 32 mm. long; Brownsville, Texas; R. D. Camp, abundant *Protoopalina ovoidea.*

No. 15681, 26 mm. long; San Diego, Texas; Thayer; no Opalinids.

* *G. usta* (Cope), No. 10021, female with eggs 1 mm. in diameter; Tehuantepec, Mexico; F. Sumichrast; many *Protoopalina xyster.*

* *Hypopachus cuneus* Cope, No. 52286, 32 mm. long; Brownsville, Texas; March 31, 1915; R. D. Camp; one bunch of Opalinids was seen but not identified.

H. pearsii Ruthven, No. 14718, 45 mm. long; Colombia, South America; May 21; no Opalinids.

* *H. variolosus* (Cope), No. 24830, 34 mm. long; Guatemala; H. Hogue; *Zelleriella hypopacheos* and cysts, probably of same species.

No. 28431, 32 mm. long; same label; no Opalinids.

No. 6792, 3 specimens, each 41 mm. long; Guatemala; H. Hogue; no Opalinids.

Kaloula picta (Eydoux and Souliet), Nos. 39157, 39159, 39162, 28 to 38 mm. long; Philippine Islands; July 10, 1908; P. Bartsch; no Opalinids.

**K. pulchra* Gray, No. 10967, 51 mm. long; Cochin China, from Museum of Natural History, Paris; numerous *Cepedea pulchra.*

Microhyla achatina Tschudi, No. 29438, 26 mm. long; female with eggs; Laut Tados, Sumatra; D. L. Karcher; no Opalinids.

M. fissipes Boulenger, No. 34469, 27 mm. long; female with eggs 1 mm. in diameter; Formosa; March, 1903; Owston; no Opalinids, 2 specimens of *Nyctotherus* seen.

M. okinavensis Stejneger, No. 23906, 27 mm. long; Kunschiau, Okinawa, Japan; I. Ijima; no Opalinids.

Nos. 34480, 34483, 34484, 27 to 32 mm. long; Ishikagi Island, Loo Choo Archipelago, Japan; April to June, 1899; Owston; no Opalinids.

**Phrynomantis bifasciata* (Smith), No. 22096, 39 mm. long; Mount Kenia, British East Africa; W. A. Chanler; many *Cepedea phrynomantidis.*

* Nos. 20113, 20115, 28 mm. long; Tana, South Africa; November, 1892; W. A. Chanler; one uninfected, the other showed many *Cepedea phrynomantidis.*

**Rhinoderma darwinii* Duméril and Bibron, Nos. 38931, 38932, 32 and 30 mm. long; Concepcion, Chile; T. Barbour; *Zelleriella darwinii* and *Protoopalina rhinodermatos.*

Rhombophryne testudo Boettger, No. 20065, 41 mm. long; Nossi Be Island, Madagascar; Edward Gerrard; no Opalinids.

Family RANIDAE.

Subfamily DENDROBATINAE.

**Dendrobates tinctorius* (Schneider), No. 32535, 39 mm. long; Costa Rica; Gabb; numerous *Zelleriella dendrobatidis.*

Nos. 32541, 32542, each 39 mm. long; same label; no Opalinids.

* Nos. 19771, 19772, each 32 mm. long; Greytown, Nicaragua; April 10, 1892; C. W. Richmond; *Zelleriella dendrobatidis.*

No. 19773, same label; no Opalinids.

Nos. 29887, 29888, each 22 mm. long; Costa Rica; Burgdorf and Schild; no Opalinids.

No. 14183, 3 specimens, each 32 mm. long; Nicaragua; August 7, 1885; Bransford; no Opalinids.

**D. typographus* Keferstein, No. 30585, 22 mm. long; Costa Rica; Gabb; numerous *Zelleriella dendrobatidis.*

No. 30590, 22 mm. long; female with a few small eggs; same label; no Opalinids.

* No. 32322, west coast Central America, *Zelleriella dendrobatidis.*

No. 32320, same label; no Opalinids.

* Nos. 19588 to 19590, each 22 mm. long; Rio San Juan, Nicaragua; February 27, 1892; Richmond; scant infections of *Zelleriella dendrobatidis.*

No. 19599, same label; no Opalinids.

* Nos. 19652, 19653, each 19 mm. long; Rio San Juan, Nicaragua; February 23, 1892; Richmond; a few *Zelleriella dendrobatidis.*

Nos. 19654, 19655, 19657, 19658, each 19 mm. long; same label; no Opalinids.

Nos. 35716, 35719 to 35721, each 22 mm. long; Nicaragua; August 7, 1885; Bransford; no Opalinids.

Subfamily RANINAE.

Astylosternus robustus Boulenger, Nos. 51411, 51412, 102 and 108 mm. long; Lolodorf, The Cameroons, west Africa; 1912; G. Schwab; no Opalinids.

Hylambates brevirostris Warner, No. 48853, 53 mm. long; The Cameroons, west Africa; Barbour; no Opalinids.

* *H. rufus* (Reichenow), No. 48850, 75 mm. long; The Cameroons, west Africa; Barbour; very many *Opalina camerunensis.*

No. 48851, 79 mm. long; same label; *Nyctotherus, Balantidium;* no Opalinids.

Hyperolius concolor (Hallowell), No. 12765, 35 mm. long; southeast Africa; British Museum; no Opalinids.

* *H. marmoratus* Rapp, No. 16053, 31 mm. long; west Africa; W. H. Brown; *Cepedea madagascariensis* [of *Hyperolius*].

Megalixalus fornasinii (Bianco), No. 57803, 25 mm. long; Ukami, German East Africa, 1889; Julius Hurter, sr.

* *M. madagascariensis* (Duméril and Bibron), No. 33878, 26 mm. long; female with eggs; Madagascar; from Zoological Institute, University of Vienna, Austria; F. Werner; *Cepedea madgascariensis.*

No. 37197, 24 mm. long; female with eggs; Madagascar; F. Werner; no Opalinids.

* *M. seychellensis* (Tschudi), Nos. 20415, 20416, 76 and 51 mm. long; Ile Mahi, Seychelle Islands, 1892; W. L. Abbott; many *Cepedea seychellensis.*

Nyctixalus margaritifer Boulenger, No. 43209, 30 mm. long; Mount Gede, Java, August, 1909; Bryant Javan Expedition; no Opalinids.

* *Oxyglossus lima* (Tschudi), No. 44022, 26 mm. long; Buitensorg, Java, March 6, 1909; *Cepedea spinifera.*

No. 38953, 35 mm. long; female with eggs; Java; no Opalinids.

No. 43990, 26 mm. long; female with eggs; Tamboen, Java; June 26, 1909; No Opalinids.

No. 7500, 39 mm. long; China; W. Stimpson; no Opalinids.

Philautus aurifasciatus (Schlegel), No. 43945, 17 mm. long; Mount Gede, Java; 4,500 feet altitude; April 21, 1909; Bryant Javan Expedition; no Opalinids.

Ph. leucorhinus (Martens), No. 12773, 2 specimens, 22 and 23 mm. long; Central Ceylon; from British Museum; no Opalinids.

Ph. variabilis (Guenther), No. 12771, 3 specimens, 18 and 23 mm. long; Himalaya Mountains; from British Museum; no Opalinids.

Phrynobatrachus acridoides (Cope), No. 20102, 16 mm. long; Tana River, British East African; November, 1892; W. A. Chanler; no Opalinids.

No. 57720, 19 mm. long; Ukami, German East Africa; 1900; Julius Hurter, sr.; no Opalinids.

* *P. natalensis* (Smith), Nos. 39477, 39479, 39481, 20, 19 and 17 mm. long; Bahr-el-Gabel, Sudan, Africa; March, 1905; F. Werner; *Opalina natalensis.*

No. 29480, 15 mm. long; same label; no Opalinids.

* *Phyllobates trinitatis* (Garman), No. 27992, 23 mm. long; La Guira, Venezuela; July 10, 1900; Lyon and Robinson; *Zelleriella* [*trinitatis*].

* No. 27806, 21 mm. long; San Julian, Venezuela; August 8, 1900; Robinson; *Zelleriella* [*trinitatis*], also *Balantidium.*

* *Polypedates buergeri* (Schlegel), No. 30745, 59 mm. long; Miyazaki, Kiusiu, Japan; C. A. Clark; a few *Cepedea buergeri.*

* No. 31905, 38 mm. long; Kochi, Shikoku, Japan; May 11; H. M. Smith; many *Cepedea buergeri.*

No. 31906, 43 mm. long; same label; no Opalinids.

* No. 23904, 42 mm. long; Province Iga, Hondo, Japan; I. Ijima; many *Cepedea buergeri.*

No. 23905, 37 mm. long; same label; no Opalinids.

P. colletti (Boulenger), No. 33147, two specimens, 45 mm. and 58 mm. long; Palo Bakong, Linga Archipelago, between Malay Peninsula and Sumatra; July 22, 1903; W. L. Abbott; no Opalinids.

P. javanus (Boettger), No. 43905, 64 mm. long, Mount Gede, Java; August, 1909; Bryant Javan Expedition; no Opalinids.

**P. leucomystax* (Gravenhorst), No. 10966, 48 mm. long; Cochin China; from Paris Museum; many *Cepedea segmentata.*

*Nos. 43906, 43907, 70 and 73 mm. long; Buitensorg, Java; March 10, 1909; Bryant Javan Expedition; a few *Cepedea segmentata.*

No. 43908, 68 mm. long; Buitensorg, Java; April 2, 1909; Bryant Javan Expedition; no Opalinids.

No. 43910, 63 mm. long, female with eggs, Buitensorg, Java, March 18, 1909, Bryant Javan Expedition; no Opalinids.

No. 44025, 58 mm. long; Buitensorg, Java; April 28, 1909, Bryant Javan Expedition; no Opalinids.

*No. 29437, 62 mm. long, female with eggs; Sumatra; L. Karscher; *Cepedea segmentata.*

*Dobell (1910) reports *Opalina virgula* from "*Rhyncophorus maculatus*" from Peradeniya, Ceylon. This is probably *Polypedates leucomystax.*

P. microtympanum Guenther, No. 12776, 23 mm. long; Ceylon; from British Museum; no Opalinids.

P. owstoni Stejneger, No. 34330, 53 mm. long; Isigaki Island, Loo Choo Archipelago, Japan; April, 1899; A. Owston; no Opalinids.

P. reinwardtii (Schlegel), Nos. 44144, 44145, 44148, 52, 53, and 73 mm. long; Buitensorg, Java; March 18, 1909; Bryant Javan Expedition; no Opalinids. The largest specimen was a female which had recently laid her eggs.

**P. schlegelii* Guenther, No. 34370, 34372, 36 and 39 mm. long; Mount Fuji, Japan; May to September, 1898; A. Owston; *Cepedea multiformis* [of *Polypedates schlegelii*].

No. 34371, 38 mm. long; same label; no Opalinids.

*Nos. 23589, 23590, 48 and 45 mm. long; one male, and one female with eggs; Yokohama, Japan; September, 1896; L. Stejneger; a very few *Cepedea multiformis* [of *Polypedates schlegelii*].

**Prostherapis boulengeri* Barbour, No. 52406, 22 mm. long; Gorgona Island, Colombia, South America; *Zelleriella* [*boulengeri*].

**Rana adspersa* Tschudi, No. 7127, 80 mm. long; female with eggs; Mozambique, Africa; W. Peters; a few *Protoopalina mossambicensis.*

*No. 42038, 72 mm. long; British East Africa; Smithsonian African expedition; a few *Protoopalina mossambicensis.*

**R. aesopus* (Cope), No. 21703, 64 mm. long; Crescent City, Florida; June 20, 1884; Henry G. Hubbard; myriads of *Opalina obtrigonoidea lata.*

R. amurensis Boulenger, No. 53370, 32 mm. long; Sungari, Manchuria, China; A. de C. Sowerby; no Opalinids (formalin?)

**R. areolata* Baird and Girard, No. 9386, 45 mm. long; northern Illinois; R. Kennicott; many *Opalina kennicotti.*

No. 38358, 78 mm. long; Montgomery County, Missouri; July 26, 1908; J. Hurter; *Balantidium*, no Opalinids.

No. 11897, 75 mm. long, female with eggs; Nashville, Tennessee; August 17, 1880; W. J. Taylor; a very few *Balantidium*; no Opalinids.

**R. arvalis* Nilson, No. 37177, 55 mm. long; Lower Austria; September, 1895; F. Werner; many *Opalina ranarum* form *arvalis.*

**R. aurora* Baird and Girard, No. 39871, 58 mm. long, female with immature eggs; Crater Lake, Oregon; August 21, 1896; Evermann and Cox, *Opalina obtrigonoidea lata.*

No. 22409, 66 mm. long; Vancouver Island, British Columbia; summer, 1893; J. Macoun; no Opalinids.

No. 39810, 80 mm. long; Tahkenitch Lake, Oregon; December 5, 1896; S. E. Meek; no Opalinids.

No. 36021, 72 mm. long, female with eggs; Seattle, Washington; M. C. Dickerson; no Opalinids.

**R. boylei* Baird and Girard. In numerous frogs of this species, purchased at San Francisco, California, and said to have been collected in the vicinity, the author found abundant *Opalina virguloidea.*

R. caeruleopunctata Steindachner, No. 29920, 33 mm. long; San Jose, Costa Rica; Burgdorf and Schild; no Opalinids.

R. camerani Boulenger, No. 37183, 44 mm. long; Cilician Taurus, Asia Minor; 1902; F. Werner; no Opalinids.

**R. cantabrigensis* Baird, No. 39757, 38 mm. long; Rapid River, Minnesota; August 9, 1884; A. J. Woelman; *Cepedea cantabrigensis.*

*No. 35510; Carberry, Manitoba; August, 1904; E. T. Silton; *Cepedea cantabrigensis.*

*No. 9383, 37 mm. long; Lake Superior; R. Kennicott; *Cepedea cantabrigensis.*

*Nos. 38552, 38553, 43 and 45 mm. long; Huron County, Michigan; A. G. Ruthven; many *Cepedea cantabrigensis*.

No. 38551; 48 mm. long; same label; no Opalinids.

**R. cantabrigensis latiremis* (Cope), Nos. 15487, 15488, 43 and 49 mm. long; females with eggs; Fort Cosmos, northern Alaska; June, 1898; P. H. Howe, jr.; *Cepedea cantabrigensis*.

* No. 33104, Nulato, Alaska; May, 1878, Nelson; a few *Cepedea cantabrigensis*.

No. 13727, 51 mm. long; female with very immature eggs; Lake Alloknagik, Alaska, June, 1898; C. C. McKay; many *Cepedea cantabrigensis*.

*No. 5924, 3 specimens, 39 and 42 mm. long; Fort Resolution, Great Slave Lake, Northwest Territory, Canada; R. Kennicott; one uninfected, two with *Cepedea cantabrigensis*, one having also *Balantidium*.

No. 6505, 40 mm. long; Big. Island, Great Slave Lake, Northwest Territory, Canada, June, 1898; John Reid; no Opalinids.

No. 6919, 32 mm. long; Fort Resolution, Great Slave Lake, Northwest Territory, Canada; no Opalinids (too soft).

**R. catesbeiana* Shaw, in 2 specimens out of 15 imported from the Hawaiian Islands (introduced) the author found *Cepedea dimidiata hawaiiensis*.

Eleven bullfrogs from Maryland were uninfected.

Seventeen young bullfrogs from Wood's Hole, Massachusetts, July and August, 1919, were uninfected, also three large tadpoles.

Five bullfrogs from Raleigh, North Carolina, April, 1915, H. H. and C. S. Brimley; no Opalinids. One had *Discophrya*.

Thirteen bullfrogs from the collections of the United States National Museum showed no Opalinids. These were as follows:

No. 3331, 76 mm. long; Sibley Lake,. Kansas; Governor Stevens.

No. 9475, 115 mm. long; St. Johns River, Florida; 1874; G. B. Goode.

No. 3690, 113 mm. long; western Missouri; R. P. Hoy.

No. 3332, 146 mm. long; St. Louis, Missouri; Lieutenant Bryan.

No. 3687, 145 mm. long; Mobile, Alabama; Pilichody.

No. 15986, 95 mm. long; Neuse River, Raleigh, North Carolina; summer 1888; D. S. Jordan.

No. 15984, 80 mm. long; Little River, Goldsborough, North Carolina; United State Fish Commission.

No. 3514, 55 mm. long; Racine, Wisconsin; S. F. Baird.

No. 8346, 40 mm. long; tadpoles; Milner.

No. 17715, 43 mm. long; tadpoles and hind legs; New Braunfels, Texas, December 3, 1891; Evermann.

No. 28376, 37 mm. long; Maxey, Tennessee, October 11; G. D. Morgan.

No. 3510, 80 mm. long; St. Lopis, Missouri; D. G. Engelmann.

No. 3336, 105 mm. long; near Shawnee Village, Arkansas River, near Fort Smith; H. B. Mollhausen.

* *R. chrysoprasina* (Cope), No. 14180, 2 specimens, 23 and 26 mm. long; Nicaragua; August 7, 1885; J. P. Bransford; *Cepedea occidentalis*.

No. 14180, another specimen, 26 mm. long; same label; no Opalinids.

No. 30653, 38 mm. long; Uren, Costa Ricao; 2,000 feet altitude; W. M. Gabb; no Opalinids.

R. clamitans Latreille. In 24 specimens from Raleigh, North Carolina, the author found no Opalinids; most contained flagellates.

In nine specimens, listed below, from the United States National Museum no Opalinids were found:

Nos. 48922 to 48924, 72, 49, and 41 mm. long; Crow Branch, Maryland; April 25, 1912; E. B. Marshall and B. A. Bean.

No. 11532, 80 mm. long; Michihicotan, Lake Superior; G. Barrister.

No. 33374, 80 mm. long; Lost Lake, Indiana; August 1, 1900; B. Evermann.

No. 3474, 85 mm. long; female with eggs; Highland County, Ohio; Mathews.

No. 39713, 67 mm. long; Pine River, Michigan; July 14, 1894; J. T. Scovell.

No. 33130, 73 mm. long; Owl Lake, near Mercer, Wisconsin; September 30, 1903; H. V. Ogden.

No. 5377, Selkirk Settlement, Donald Guren.

* In tadpoles, probably of this species, from Chester, Nova Scotia, occur *Opalina* [*larvarum*], collected by Dr. E. Simon in the summer of 1920.

* *R. copei* Boulenger, Nos. 30654, 30655, 42 and 27 mm. long; Pico Blanco, Costa Rica; W. M. Gabb; *Opalina copei.*

No. 12289, 40 mm. long; Yucatan; Schott; no Opalinids.

* *R. crassipes* Buchholz and Peters, No. 48852, 55 mm. long; Kribi, The Cameroons, west Africa; from the Museum of Comparative Zoology, Cambridge, Massachusetts; numerous *Protoopalina africana.*

* *R. cyanophlyctis* Schneider, "Asia," *Opalina coracoidea*, reported by Bezzenberger (1904).

* *R. dalmatina* Fitzinger, No. 37181, 70 mm. long; female with eggs; Travnik, Bosnia; 1893; E. Brandis; *Opalina ranarum.*

* No. 34552, Lugano, Switzerland; June, 1904; L. Stejneger and G. S. Miller; *Opalina ranarum.*

No. 11068, 59 mm. long; Turin, Italy; from Royal Zoological Museum; no Opalinids (poor condition, soft).

* No. 11895, 60 mm. long; "Europe;" E. G. Blackford; from Liverpool Aquarium; myriads of *Opalina ranarum.*

* No. 11895, another specimen, 52 mm. long; same label; *Opalina ranarum.*

* *R. draytonii* Baird and Girard. Of numerous frogs of this species purchased in San Francisco, California, and said to have been collected in the vicinity, one contained *Zelleriella ranaxena*, and two others had *Opalina draytonii.*

Four specimens from south of San Francisco, California, contained no Opalinids.

Of 22 specimens examined at Friday Harbor, Puget Sound, Washington, none had Opalinids. The locality from which these were collected was not known. They were in captivity over a month before they were examined.

* No. 32721, San Francisco, California; May to June, 1865; Dall, Pease, and Ketchum; *Opalina draytonii.*

No. 20379, Witch Creek, California; May, 1893; H. W. Henshaw; no Oplinids.

No. 8700, Fort Tejon, California; August, 1875; H. W. Henshaw; no Opalinids.

* Nos. 23668, 23669, 79 and 80 mm. long; San Pedro Mountains; Lower California, Mexico; 1889; C. H. Townsend; *Opalina draytonii.*

No. 23670, 76 mm. long, same label; no Opalinids.

No. 9420, Puget Sound; C. B. R. Kennerly; no Opalinids.

No. 33574, 60 mm. long; Presidio, California; Lieut. W. P. Trowbridge; no Opalinids.

R. emeljanovi Nikolski, No. 52357, 55 mm. long; female with eggs; Tientsin, China; A. de C. Sowerby; no Opalinids.

No. 52359, 31 mm. long, same label; no Opalinids.

R. erythraea (Schlegel), No. 53518, 28 mm. long, Ok Yam, Siamese Cambodia boundary, January, 1915, C. Boden Kloss, a few *Opalina rotunda*.

No. 38956, 30 mm. long; Buitensorg, Java; Thomas Barbour; no Opalinids.

No. 43924, Depok, Java, June 14, 1909, Bryant Javan Expedition, no Opalinids (formalin ?).

**R. esculenta* Linnaeus, Different observers have reported from the European "edible frog" *Protoopalina intestinalis, Cepedea dimidiata* and its form *zelleri.*

* Galli-Valerio (1907) reports *Opalina ranarum* from the Swiss Canton Vaud.

* Andre (1913) reports *Opalina ranarum* from Switzerland.

* "*R. esculenta chinensis,*" "Asia," is reported by Bezzenberger (1904) to contain *Cepedea lanceolata*. This is apparently *Rana nigromaculata.*

* *R. esculenta hispanica* Michahelles, Nos. 38481 to 38483, 48, 47, and 38 mm. long; Alicante Province, Spain; January 25, 1907; Thomas and Miller; many *Cepedea hispanica.*

**R. esculenta ridibunda* (Pallas), Nos. 49839 (68 mm. long, female with eggs), 49840 (80 mm. long, female with small eggs), 49848 (70 mm. long, male), Biskra, Sahara, May 26, 1912, D. D. Streeter, jr., no Opalinids.

* No. 49841 (85 mm. long, female with eggs); same label; many *Cepedea saharana.*

* Nos. 49842 (85 mm. long, female with small eggs); 49843 (88 mm. long, female with eggs); same label; the former with a few; the latter with a very few *Cepedea saharana.*

* No. 49845, 88 mm. long; female with small eggs; same label; a few *Cepedea saharana.*

R. forreri Boulenger, No. 3365, 60 mm. long; Chihuahua, Mexico; C. B. R. Kennerly; no Opalinids.

R. grayi (?) Smith, Nos. 16092, 16094, 43 and 28 mm. long; St. Helena Island, Africa; February 22, 1890; A. H. Brown; no Opalinids.

R. grylio Stejneger. No Opalinids are known from the southern bullfrog. Specimens have been examined as follows:

No. 3688, 2 specimens, 105 and 108 mm. long; Pensacola, Florida; Dr. Jaffrey.

Nos. 27446, 27447, 94 and 75 mm. long; Bay St. Louis, Mississippi; G. S. Miller.

No. 28858, 45 mm. long; Lake Kissimee, Florida, March 18, 1901; E. A. Mearns.

Nos. 29010, 29011, 160 and 70 mm. long; Kissimee, Florida, May 4, 1902; E. A. Mearns; many *Nyctotherus.*

No. 30951, 150 mm. long; female with very immature eggs, Lemon City, Florida; Brown.

No. 29007, 95 mm. long; Kissimee, Florida, May 4, 1902; E. A. Mearns.

**R. japonica* (Guenther), Nos. 31907, 31912, 48 and 36 mm long; Kochi, Shikoku, Japan; May 11; H. M. Smith; *Opalina japonica.*

No. 31911, 37 mm. long; same label; no Opalinids.

R. latastei Boulenger, No. 37178, 26 mm long; Gorizia, Austria; September, 1906; F. Werner; no Opalinids.

* *R. limnocharis* Wiegmann. Bezzenberger (1904) reports from "Asia" *Cepedea longa* and *Opalina lata.*

* No. 31798, 50 mm. long; female with eggs; Onomichi, Japan; June 4; H. M. Smith; *Cepedea longa.*

* No. 36552, 35 mm. long; Okinawashima, Japan; from Science College, Tokyo; *Cepedea longa.*

* No. 36502, 41 mm. long; Giilan, Formosa; October, 1896; T. Tada; many *Cepedea longa.*

* No. 23902, 35 mm. long; Kumamoto, Kiusiu, Japan; March 3, 1884; I. Ijima; *Cepedea longa.*

No. 30744, 24 mm. long; Miyazaki, Japan; C. A. Clark; no Opalinids.

No. 35273, 33 mm. long; Nagasaki, Japan; E. A. Mearns; no Opalinids.

No. 38123, female with eggs; Yamagawa, Japan; H. M. Smith; no Opalinids.

No. 36501, 46 mm. long; Taipa, Formosa; September, 1896; T. Tada; no Opalinids.

*No. 44158, 35 mm. long; Mount Gede, Tjibodas, Java; August, 1909; Bryant Javan expedition; *Opalina japonica* (?), also *Balantidium.*

No. 7496, China, W. Stimpson; no Opalinids.

No. 12769, 37 mm. long; Ceylon; from British Museum; no Opalinids.

No. 12772, 2 specimens, 38 and 27 mm. long; China; from British Museum; no Opalinids.

**R. macrodon* Tschudi, No. 43931, 75 mm. long; Mount Salok, Java; May, 1909; Bryant Javan expedition; a few *Protoopalina quadrinuclcata,* also *Balantidium.*

**R. mascareniensis* Duméril and Bibron, No. 33112 or 33113 (both labels half destroyed), 43 mm. long; Gold Coast, West Africa; *Opalina* species (?).

No. 33113, or 33112, 45 mm. long; same label; *Balantidium,* no Opalinids.

No. 57671. 36 mm. long; Mauritius, Africa; 1897; Julius Hurter, sr.; no Opalinids.

R. montezumae Baird, No. 16562; Orizaba, Mexico; F. Sumichrast; no Opalinids.

No. 25099, 76 mm. long; female with eggs; Mirador (?), Mexico; Doctor Sartorius; no Opalinids.

No. 9891, 90 mm. long; female with eggs; Guanajuato, Mexico; 1887; A. Dugés; no Opalinids.

No. 26155, 84 mm. long; Guanajuato, Mexico; A. Dugés; no Opalinids.

**R. nigromaculata* Hallowell, No. 30739, 60 mm. long; Miyazaki, Japan; C. A. Clark; a few *Cepedea dimidiata orientalis.*

*No. 23579, 61 mm. long; Yokohama, Japan; September, 1896; L. Stejneger; very many *Cepedea dimidata orientalis.*

No. 23584, 49 mm. long; same label; a few *Balantidium;* no Opalinids.

No. 21201, 69 mm. long; Seoul, Korea; 1883; Jouy; no Opalinids.

No. 7415, 70 mm. long; Simode, Hondo, Japan; W. Stimson; no Opalinids (too soft).

No. 8852, 2 specimens, each 50 mm. long; Yokohama, Japan; May, 1874; no Opalinids (soft).

No. 22218, 48 mm. long; Japan; no Opalinids (soft).

No. 31938, 60 mm. long; Kochi, Shikoku, Japan; May 11; H. M. Smith; no Opalinids (formalin?).

No. 34468, 41 mm. long; Yokohama, Japan; Feb. 4, 1901; A. Owston; no Opalinids.

*No. 39352, 60 mm. long; Yen-An-Fu, Shensi, China; August 24, 1909; A. de C. Sowerby, *Protoopalina axonucleata lata.*

*No. 39351, 20 miles east of Hai Shen SSu, Shensi, China; August 17, 1909; A. de C. Sowerby; abundant *Protoopalina axonucleata lata.*

No. 39353, 73 mm. long; same label; no Opalinids.

*From "*Rana esculenta chinensis,*" which is apparently *Rana nigromaculata,* Bezzenberger (1904) reports *Cepedea lanceolata.*

**R. nutti* Boulenger, from German East Africa; Awerinzew (1913); reports *Protoopalina primordialis.*

No. 39436, 38 mm. long; Kisbosho, German East Africa; J. Roux; no Opalinids.

*Of nine specimens collected by The Smithsonian African Expedition, on "the Mount Kenia trip," in British East Africa, October, 1909, all but one were infected. The records are as follows:

*No. 41434, 56 mm. long; female with large eggs; many *Protoopalina nutti.*

*No. 41435, 63 mm. long; female with eggs 1½ mm. in diameter; a few *Protoopalina nutti.*

*No. 41438, 51 mm. long, very many *Protoopalina nutti*, also *Balantidium.*

*No. 41439, 65 mm. long; female just after ovulation; exceedingly abundant *Protoopalina nutti*, also *Nyctotherus.*

*No. 41441, 45 mm. long, 2 specimens; one uninfected, one showed many *Protoopalina nutti.*

*No. 41444, 55 mm. long, female with a few large eggs, 2 specimens; one gave a few, the other bore very many *Protoopalina nutti.*

*No. 41445, 48 mm. long, female with a few large eggs; myriads of *Protoopalina nutti*, also *Balantidium.*

R. onca Cope, No. 18959, 62 mm. long; female captured during ovulation; Vega Valley, Nevada; March 18, 1891; E. W. Nelson; no Opalinids.

No. 18958, 56 mm. long; same label; no Opalinids.

No. 18960, 65 mm. long; northern Mexico; March 9, 1891; E. W. Nelson; no Opalinids (soft).

R. *oxyrhynchus* Sundevall, No. 57521, 35 mm. long; German East Africa; Julius Hurter, sr.; no Opalinids.

R. palmipes Spix, Nos. 24811, 24813, 75 and 44 mm. long; Guatemala; H. Hogue; no Opalinids (soft). The larger of these frogs had the wall of the caecal region calcified.

No. 25231, 66 mm. long; Guatemala; Van Patten; no Opalinids (soft).

Nos. 30329, 30331, 59 and 43 mm. long; Tehuantepec, Mexico; F. Sumichrast; no Opalinids (soft).

**R. palustris* LeConte. In many living frogs from Woods Hole, Massachusetts, and Oberlin, Ohio, the author has found *Opalina obtrigonoidea* and its form *plicata.*

*Three living specimens; Raleigh, North Carolina; April, 1915; H. H. and C. S. Brimley; 2 were uninfected, 1 had abundant *Opalina obtrigonoidea.*

* No. 3434, 47 mm. long; West Philadelphia, Pennsylvania; W. S. Wood; *Opalina obtrigonoidea*

*No. 3406, 68 mm. long; Detroit River, Michigan; August, 1853; S. F. Baird; *Opalina obtrigonoidea.*

*No. 9388, 50 mm. long; upper Wisconsin River; Hammond; *Opalina obtrigonoidea.*

No. 3408, 63 mm long; Framingham, Massachusetts; S. F. Baird; no Opalinids.

No. 39817, 53 mm. long; Rocky River, Olmstead Falls, Ohio; June, 1893; A. J. Woolman; no Opalinids.

No. 3407; Washington County, Mississippi; D. L. C. Wailes; no Opalinids.

**R. pipiens* Schreber, No. 3295, 72 mm. long; labeled *Rana utricularia;* Charco Escondino; Matamoros, Tamaulipas, northeastern Mexico; March, 1853; Couch; many *Cepedea mexicana.*

No. 3295, 2 other specimens, 47 and 68 mm. long; same label; no Opalinids.

*In numerous frogs from Oberlin, Ohio, Raleigh, North Carolina, and Chicago, Illinois, the author has found *Opalina obtrigonoidea.*

*No. 3413, 90 mm. long; Port Huron, Michigan; S. F. Baird; *Opalina obtrigonoidea.*

*No. 9257, 2 specimens, 54 and 45 mm. long; Washington, District of Columbia; June 10, 1877; T. H. Bean; the smaller was uninfected, the larger contained a few *Opalina obtrigonoidea.*

No. 33238, 35 mm. long; Lake Maxinkuckee, Indiana; July 8, 1899; United States Fish Commission; no Opalinids (formalin?).

No. 25146, 65 mm. long; Arlington, Virginia; April 8, 1898; William Palmer; no Opalinids.

No. 11476, 60 mm. long; female with eggs; labeled *Rana halecina hybridea;* Waukegan, Illinois; James Millner; no Opalinids.

**R. pipiens austricola* (Cope), No. 6506, 2 specimens, 38 and 55 mm. long; Tobasco, Mexico; "in wells"; March, 1864; Dr. Berendt; *Opalina obtrigonoidea austricola.*

*No. 24809, 42 mm. long; Guatemala; H. Hogue; a few *Opalina obtrigonoidea austricola.*

No. 28405, 65 mm. long; same label; no Opalinids.

*No. 30554, 27 mm. long; Pico Blanco, Costa Rica; W. M. Gabb; many *Opalina obtrigona austricola.*

No. 30555. 25 mm. long; same label; no Opalinids.

**R. pipiens sphenocephala* (Cope), No. 29003, 83 mm. long; Kissimee Prairie, Florida; April 22, 1901; E. A. Mearns; very many *Opalina carolinensis.*

No. 29004, 91 mm. long; female in ovulation; same label; no Opalinids.

*Eight specimens from Raleigh, North Carolina; April, 1915; H. H. and C. S. Brimley; six uninfected, two abundantly infected with *Opalina carolinensis.*

*No. 3425, 58 mm. long; female with eggs; Charleston, South Carolina; C. Girard; many *Opalina carolinensis.*

No. 3425, another specimen. 45 mm. long; same label; no Opalinids.

No. 5192, 60 mm. long; New Orleans, Louisiana; St. Charles; no Opalinids.

No. 26313, 43 mm. long; Lemon City, Florida; E. J. Brown; no Opalinids.

No. 3436. Tarboro, North Carolina; Bridger; no Opalinids.

No. 39865, 53 mm. long; locality?; no Opalinids.

No. 10312, 72 mm. long; Oakley, South Carolina; May, 1879; F. W. Haywood; no Opalinids.

No. 2428, Pensacola, Florida; Hammond; no Opalinids.

**R. pretiosa* Baird and Girard, Nos. 17587, 17588, each 72 mm. long; Elliston, Montana; July 21, 1891; B. W. Evermann; many *Opalina copei.*

*No. 17603. 63 mm. long; Deer Lodge River, Montana; July 22, 1891; B. W. Evermann; *Opalina copei.*

No. 17617, 46 mm. long; National Park, Wyoming; August 8, 1891; B. W. Evermann; no Opalinids.

No. 13770, Deschutes River, Washington; Bendire; no Opalinids.

* *R. rugosa* Schlegel. No. 31802. 33 mm. long; Yamoto River, Nara, Japan; H. M. Smith; *Cepedea pulchra japonica.*

*Nos. 31813, 31814 length 45 mm. (female in ovulation) and 35 mm.; Koriyama, Japan; April 26; H. M. Smith; *Cepedea pulchra japonica.*

No. 22190, 40 mm. long; Japan; Moore; no Opalinids.

**R. septentrionalis* Baird, No. 13622, 45 mm. long, Lucknow, Ontario, Canada, 1883; J. H. Garnier, a few *Opalina species* (?).

No. 39792, 55 mm. long; White Oak Lake, Deer River, Minnesota; August 21, 1894; R. J. Woolman; no Opalinids.

No. 36466, 38 mm. long; First Connecticut Lake, New Hampshire; July 21, 1904; no Opalinids.

No. 42109, 40 mm. long; Michigan (?); Powell; no Opalinids.

No. 3437, 67 mm. long; North Red River, North Dakota; R. Kennicott; no Opalinids.

No. 39814, 31 mm. long; Clyde River, Newport, Vermont; July 23, 1894;

No. 5366, Moose River, British America; C. Drexler; no Opalinids.

Evermann and Bean; no Opalinids.

No. 5379, 65 mm. long; female just after ovulation; Selkirk Settlement; R. Kennicott; no Opalinids.

No. 3432, 49 mm. long; Madrid, New York; S. F. Baird; no Opalinids.

Nos. 39746 to 39748, 48, 35, and 29 mm. long; Mill Creek, Jacket Harbor, New York; July 22 1894; Evermann and Bean; no Opalinids.

No. 13605, 2 specimens, 53 and 60 mm. long; Lucknow, Ontario, Canada; July, 1883; J. H. Garnier; no Opalinids.

No. 28453, 48 mm. long; Peterboro, New York; October 25, 1900; Mrs. E. P. Miller; no Opalinids.

*_R. sylvatica_ LeConte. In numerous living specimens from Oberlin, Ohio, and Raleigh, North Carolina, the author has found _Opalina virguloidea_. About two-thirds of the specimens were infected.

R. temporalis Guenther, No. 12774, 21 mm. long; Anamallays, southern India; from the British Museum; no Opalinids.

*_R. temporaria_ Linnaeus. From this European wood frog many observers have reported _Opalina ranarum_. André (1913) reports from Switzerland _Cepedea dimidiata_ and _Protoopalina intestinalis_, (the latter observed in but a single host).

*No. 28274, 44 mm. long; Sapporo, Japan; 1906; United States Bureau of Fisheries; _Opalina ranarum_.

No. 23438, 59 mm. long; Sapporo, Yezo Island, Japan; S. Vozawa, no Opalinids.

*_R. temporaria parvipalmata_ Sevane. No. 38487, 35 mm. long, Ariege, France. Thomas and Miller, many _Opalina ranarum_ form _parvipalmatae_.

*No. 38485, 80 mm. long; female with eggs; Porté, France; August 31, 1906; Thomas and Miller, _Opalina ranarum_ form _parvipalmatae_.

*_R. tigerina_ Daudin, No. 35295, 68 mm. long, Billiton Island, near Sumatra, August 8, 1904, W. L. Abbott, myriads of _Cepedea ophis_ in the small intestine, none in the rectum.

*No. 35296, 65 mm. long; same label; rectum already destroyed; 2 individuals of _Cepedea ophis_ found in the rectal end of the small intestine.

*No. 35297, same label; myriads of _Cepedea ophis_ in the rectum.

*No. 44006, 38 mm. long; Java; Bryant Javan expedition; a few _Cepedea ophis_.

*Nos. 38217, 38219, 42 and 69 mm. long; Kagi, Formosa; September 1 to 20, 1907; H. Sauter; a few _Protoopalina filiformis_.

No. 7435, 83 mm. long; Hongkong, China; W. Stimpson; no Opalinids.

No. 37512, 85 mm. long; Hongkong (?), China; 1881; P. L. Juoy; no Opalinids.

R. tsushimensis Stejneger, No. 17,520, 35 mm. long; Tsushima Island, Japan; May, 1895; Juoy; no Opalinids.

R. virgatipes Cope, No. 37851, 47 mm. long; Lakehurst, New Jersey; June 29, 1907; W. T. Davis; no Opalinids (formalin ?).

Nos. 36629, 36631, 38 and 33 mm. long; Lake Ellis, North Carolina; May 11, 1906; Brimley; no Opalinids.

No 22802, 48 mm. long; Lake Drummond, Virginia; June 6, 1896; W. Palmer; no Opalinids.

Rappia vermiculata Peters. No. 57522, 25 mm. long; Uganda, British East Africa; Julius Hurter, sr.; no Opalinids.

"*Rhyncophorus maculatus*" (probably *Polypedates leucomystax*) from Peradeniya, Ceylon. Dobell reports *Opalina virgula*.

We have referred incidentally to the susceptibility of different families of Anura to infection by different groups of Opalinidae. There is no sharply defined specificity of infection, though there is clear evidence of a broader grouping of hosts and parasites.

The Discoglossidae contain *Protoopalinae* of the five species *caudata*, *intestinalis*, *orientalis*, and *macrocaudata;* and one species, *Discoglossus pictus* is known to contain a *Cepedea.* This family. then, harbors the more primitive of the binucleated and of the multinucleated Opalinidae. There is also a single report of *Opalina ranarum* in *Bombina bombina.* The Discoglossidae have never come into contact with *Zelleriella.*

The Pelobatidae harbor a wider range of Opalinid parasites. The European *Pelobates fuscus* contains *Protoopalina pelobatidis*, a species of the archaic *intestinalis* group, and the Javan *Megalophrys montana* bears a somewhat similar species, *Protoopalina montana.* The different species of *Scaphiopus*, all American, are known to bear nine species of Opalinids, three of these being *Protoopalinae* (*hammondii*, *mexicana*, *scaphiopodos*) all with midmitotic nuclei; two being *Zelleriellae* (*couchii*, *scaphiopodos*); one being a *Cepedea* (*floridensis*), and three being members of the group of narrow species of the genus *Opalina*, all genera of the Opalinidae thus being known from the Pelobatidae. Only the *Opalinae latae* are unreported. It seems that the Pelobatids are hospitable to any of the groups of Opalinids, and possibly the only reason we know no broad Opalinas from these hosts is because there are so few records of examination of Pelobatids from the palaearctic habitat of the broad Opalinas. Not only does the family Pelobatidae harbor all genera of Opalinids; the same is true of the genus *Scaphiopus.*

The Bufonidae, a wide-spread family, contain all genera of Opalinidae and both the narrow and the broad species of the genus *Opalina.* They apparently collect whatever sorts of Opalinid are present in their habitat.

The Hylidae, a family found in tropical America and Australia, some of whose members have spread to North America and through North America to the northern parts of the Eastern Hemisphere. show representatives of all genera of Opalinidae, but lack broad species of the genus *Opalina.* Their Protoöpalinas are all of the less modified species and are reported only from Australia. Their Zelleriellas are from Hylas from Paraguay, Venezuela, and the West

Indies. Their Cepedeas are from Central America or western Brazil. Their narrow Opalinas are from North and Central America, except for the one species, *obtrigona*, which has spread to Asia and Europe and three (four?) derived and closely related species in Asia and Abyssinia (?). The Hylidae are resistant to infection by *Zelleriella*, for but three of the many species of *Hyla* examined are known to have adopted these parasites, though the latter are abundant in the habitat of the Hylas. They are known to carry *Protoopalina* only in Australia, though they may be in contact in South America with a species of *Protoopalina* closely related to the species which live in the Australian Hylas. Also *Hyla arborea*, of temperate Asia and Europe, has come into contact with another species of *Protoopalina* similar to one of the species found in an Australian *Hyla*. Some of the Central American and North American Hylas live in the same region with the genus *Scaphiopus* whose species bear the more highly evolved members of the genus *Protoöpalina*. The characteristic parasites of the Hylidae are the *Opalinae angustae*. These are wholly Central American and North American, except for the one migrant species in Asia and Europe. America, especially tropical America, shows the greatest development of the Hylidae, for it is here only that most of the genera of the family are found, all but one of the genera of Hylids being known from tropical America and only four of these having any representatives outside this area. The only genus of Hylid not found in tropical America is the eastern North American *Acris*.

The Leptodactylidae bear Protoöpalinas and Zelleriellas, but not any of the multinucleated species. The Australian genera *Limnodynastes* and *Uperoleia* bear *Protoöpalina acuta*, *P. dorsalis*, *P. peronii*, and *P.* "*intestinalis*" which are archaic species. The Patagonian *Eleutherodactylus leptopus* bears the closely similar *Protoopalina diplocarya*. The Ecuadortan *Telmatobius jelskii* bears the quite distinct *Protoopalina longinucleata*. The long, slender *Protoopalina tenuis* is found in the Australian genera *Crinia* and *Uperoleia*. The Australian genus *Limnodynastes* and the tropical American genera *Eleutherodactylus*, *Leptodactylus*, *Paludicola*, and *Telmatobius* harbor *Zelleriellae* all so similar that species demarcations in some cases are not easy to determine. The Leptodactylids have not come much into contact with the multinucleated Opalinidae. In America they are a tropical and south temperate family, while the multinucleated Opalinidae, especially the Opalinas, are more northern, but both *Cepedea* and *Opalina* are present in the more northern parts of the habitat of the Leptodactylidae. Five Hylidae in Central America harbor *Opalinae*, while three harbor *Cepedeae*. One *Hyla* in Paraguay and one in northern Brazil carry *Cepedea*. We have already seen that the Hylidae are reluctant to accept the characteristic Leptodactylid parasites, the Zelleriellas, though being in the same region. We have also seen that the Leptodactylids are completely resistant to infection by the Hylid parasites, the Cepedeas and Opalinas, though they live in contact with them.

But the Hylidae are not the only Anura in tropical and south temperate America which carry multinucleated Opalinids. One *Rana* (*chrysoprasina*) in Central America harbors *Cepedea occadentalis* and two other Central American *Ranae* (*copei* and *pipiens austricola*) bear *Opalinae* (*copei* and *obtrigonoidea austricola*). The Bufos also, from the same habitat, contain both Cepedeas and Opalinas. *Bufo haematiticus*, of Central America, bears *Cepedea*, and this *Bufo* and its neighbor *B. typhonius* bear respectively an as yet unnamed *Opalina* and *O. panamensis.* The Ranas have been almost as unwilling to accept Zelleriellas from the Leptodactylids as the latter have been to accept multinucleated Opalinids from the Ranas. Only one *Rana*, so far as known, bears *Zelleriella*, while no Leptodactylid is known to bear any member of the subfamily *Opalininae.* But we must remember that few Ranas have come into contact with *Zelleriella.* The Bufos, on the other hand, have been most hospitable to the Leptodactylid Zelleriellas, adopting them freely, but the Leptodactylids have refused to share with the Bufos their entertainment of the multinucleated parasites.

The Gastrophrynidae are well represented in tropical, south temperate, and north temperate America, in tropical Asia and the East Indies, on the African Continent, and are especially numerous in genera in Madgascar. I have Opalinids from only 10 American, 1 African, and 1 Cochin China species. The parasites of southern Asian and especially Madagascaran species should be thoroughly studied. They will probably yield valuable evidence as to the remarkable relations indicated between the Anura of these two regions. Papuan species should also have their Opalinids studied for comparison with the parasites, on the one hand, of American Gastrophrynidae and, on the other hand, of East Indian species of this family. I realize fully the inadequacy of the data here presented, but have no fuller material available. Two species of *Gastrophryne* from Texas and southern Mexico give two Protoöpalinas of quite a distinct group containing only the species *ovoidea* and *xyster.* The latter is the species of *Protoopalina* which approaches nearest to *Zelleriella.* The anterior portion of its body is flattened. Further flattening affecting the rest of the body would produce a very good *Zelleriella.* Another *Protoopalina* of a different sort is found in *Rhinoderma darwinii* from Chile. Four Central American Gastrophrynids of the genera *Atelopus*, *Hypopachus*, and *Engystomops*, the latter of which extends into the West Indies, harbor *Zelleriella*. and one *Atelopus* from Paraguay and a *Rhinoderma* from Chile also carry *Zelleriella*. In the eastern United States we have *Gastrophryne carolinensis* which carries a narrow *Opalina* (*obtrigonoidea*). The southern African *Phrynomantis bifasciata* carries a *Cepedea* (*phrynomantidis*), and *Kalvula pulchra* from Cochin China also carries a *Cepedea* (*pulchra*). Without more data, including especially infection records from Madagascar and India, it is hardly worth while to discuss in their broader significance the Opalinid parasites of the family Gastrophrynidae.

The Ranidae in my material are mostly of the sub-family *Raninae*, though I have two species of *Dendrobatinae* also. It is to be regretted that more material from tropical Asia and tropical Africa is not included. Only the genus *Rana*, in this family, is known to harbor *Protoopalina: R. nutti* from East Africa carries *P. nutti* and also *P. primordialis*, which from Awerinzew's description seems probably the most primitive *Protoopalina* known. At the other extreme *Rana macrodon* of Java carries *Protoopalina quadrinucleata* and the Chinese *R. nigromaculata* carries *P. axonucleata lata*, these species being, in nuclear condition, the most highly developed of the *Protoopalinae*. *Rana esculenta* of Europe is said to carry *Protoopalina interstinalis*. *Rana adspersa* of eastern Africa carries the more modified species *Protoopalina mossambicensis*. Two elongated *Protoopalinae* (*africana* and *filiformis*) are borne respectively by *Rana crassipes* from The Cameroons and *R. tigerina* from Formosa and the East Indies. Only one *Rana* (*draytonii*) is known to bear a *Zelleriella* (*ranaxena*). The record is from California, but this species of *Rana* extends into Mexican California, which is an approach to the Central American region where *Zelleriellae* are abundant. The only other *Zelleriellae* ("*trinitatis*" and *boulengeri* [?]) borne by members of the sub-family *Raninae* are carried respectively by *Phyllobates trinitatis* from Venezuela and *Prostherapis boulengeri* of Colombia, South America. Five genera of *Raninae* are known to harbor *Cepedea*. Of these there are *Ranae* from North America (2 species), eastern Asia (4 species), Europe (2 species), northern Africa (1 species), Hawaii (1 species, introduced), the East Indies (1 species), and from Central America (1 species). The other genera infected with *Cepedea* are *Polypedates* from eastern Asia (2 species), southeastern Asia and the East Indies (1 species), *Megalixalus* from Madagascar (1 species), and the Seychelles (1 species), *Hyperolius* from West Africa (1 species) and *Oxyglossus* from Java (1 species). Twenty-six species and subspecies of *Rana* bear *Opalinae*. Of these the Eastern Hemisphere Ranas bear broad Opalinas and the Western Hemisphere species, with perhaps three exceptions, bear narrow Opalinas. The exceptions are *Rana draytonii* of California (bearing *Opalina draytonii*) and *R. copei* and *R. pretiosa* of Costa Rica and of Montana respectively (bearing the rather broad form *O. copei*). Species of other genera of *Raninae* bearing *Opalinae* are *Hylambates rufus* of West Africa (bearing *O. camerunensis*), *Phrynobatrachus natalensis* of the Sudan (bearing *O. natalensis*) and *Polypedates maculatus* of Ceylon (bearing *O. virgula*, a narrow form which, however, seems not to belong to the same group as the narrow *Opalinae* from the Western Hemisphere).

We thus see that the *Raninae* bear 6 species of *Protoopalina*, 3 species of *Zelleriella*, 18 species and subspecies of *Cepedea*, and 19 species and subspecies of *Opalina*. The multinucleated Cepedeas and Opalinas are therefore the characteristic Opalinids of the *Raninae*.

I have had for study two species of one of the three genera of the subfamily *Dendrobatinae*. These are *Dendrobates tinctorius* and *D. typographus*, both from Central America and both bearing *Zelleriella dendrobatidis*. No material has been available of *Mantella* from Madagascar, or of *Cardioglossa* from the French Congo.

From this review the Ranidae are seen to bear all the genera and subgenera of Opalinidae, though few species are infected with *Zelleriella*, apparently in part because few species of Ranidae live in the region where *Zelleriella* occurs. They seem hospitable to any of the Opalinidae except *Zelleriella* with which they come into contact and they have proven able to adopt *Zelleriella*.

There are three further inquiries we may answer from this list of infection data: First, as to the frequency of infection of Anura by Opalinids; second, as to the species of Anura which are known to harbor more than one species of Opalinid; and third, as to the simultaneous infection of one individual Anuran by more than one species of Opalinid.

First.—How frequent are infections by Opalinidae in the several species of Anura? What proportion of the individuals are infected? The material from the United States National Museum can hardly be used to answer this question, for it is not certain what specimens were promptly preserved after capture and were put in alcohol. Specimens left to die before being preserved, or even specimens kept in captivity too long, may not show Opalinidae, though these may have been present originally. Again, specimens preserved in formalin rarely show Opalinids. Not only the parasites but also the epithelial lining of the rectum are generally degenerate in formalin specimens. I shall not attempt to pick out from the list the data from specimens apparently preserved in alcohol, because, at best, one could not be certain of this, since in many instances specimens originally in formalin have been transferred to alcohol. For the most part, then, we will note only the living Anura which were searched for Opalinidae.

PISCES.

Box boops: Leger and Duboscq (1904, *b*) report that all adults at Cavalière and at Cannes were infected. At Banyuls three-fifths of the young fish, 7 centimeters long, were infected.

URODELA.

Ambystoma opacum: Several living specimens, all uninfected.

A. maculatum (*punctatum*): 5 living specimens, all uninfected.

A. tigrinum: 7 living specimens, all uninfected. Professor Powers, the discoverer of *Protoopalina mitotica*, writes me implying that this species of host, at Lincoln, Nebraska, is frequently infected by this *Protoopalina*.

Desmognathus fuscus: 5 living specimens, all uninfected.

Eurycea rubra (*Spelerpes rubrum*): 6 living specimens, all uninfected.

Plethodon glutinosus: 3 living specimens, all uninfected.

Triturus alpestris: A single report, Galli-Valerio (1907), of infection of this species by *Opalina ranarum*.

T. vulgaris (*Triton taeniatus*): Infection of this species has several times been reported by European zoölogists, but such infection is rare.

Notophthalmus viridescens (*Diemictylis viridescens*): 17 living specimens, all uninfected.

Necturus maculosus: 2 living specimens, both uninfected.

We see that three species of Urodela are known to harbor Opalinidae. As *Triturus* is only rarely infected, its infection may be but transient and due to the fact that *Triturus* feeds upon tadpoles of Anura which regularly carry Opalinidae. However, doubt is cast upon this suggestion by the fact that no Opalinids have been reported from other species of *Triturus*, or from the American *Notophthalmus* (*Diemictylis*), which have similar feeding habits.

ANURA.

Family PIPIDAE.

We have no reports from examination of living members of this family.

Family DISCOGLOSSIDAE.

Bombina bombina (*Bombinator igneus*): Of 63 living specimens examined, 25 contained *Protoopalina caudata*, 15 contained *P. intestinalis*, 23 contained no Opalinids; that is, about two-thirds were infected.

Bombina pachypa (*Bombinator pachypus*): Of 105 living specimens examined, 61 bore *Protoopalina caudata*; 34 bore *P. intestinalis*; 1 diseased host bore distorted *Protoopalinae*, which may have been *P. caudata* or *P. intestinalis* or possibly (?) both species; 8 bore no Opalinids; that is, eleven-twelfths of the individuals were infected.

The Bombinas are thus seen to be usually infected.

Family PELOBATIDAE.

Scaphiopus solitarius: Of 4 living specimens, 3 carried *Opalina obtrigonoidea.*

Family HYLIDAE.

Acris gryllus: Of 11 living specimens, 7 bore *Opalina virguloidea magninucleata.*

Chorophilus feriarum: Of 9 living specimens, 3 had *Opalina chorophili* and 6 were uninfected.

Ch. triseriatus: Many living specimens from three localities examined, infection being usual. This statement is made from memory, as I have not the notes on the observations.

Hyla arborea: Of 49 living specimens, 21 were infected with *Opalina obtrigona,* 28 being uninfected.

H. pickeringii: Many living specimens were examined. The notes of the examinations are mislaid. From memory I would say less than half were infected with *Opalina obtrigonoidea.*

H. versicolor: 2 living adults from Massachusetts were uninfected; one half grown individual from Michigan was infected with *Opalina hylaxena;* several living adults from Ohio were uninfected; 7 living larvae from Massachusetts were all infected with *Opalina hylaxena* form *orbiculata* or form *parvinucleata.*

Family BUFONIDAE.

Bufo bufo (*B. vulgaris*)*:* 1 living specimen, examined by Metcalf, bore no Opalinids. Andre (1912, *b*) reports that of these toads from Switzerland 48.7 per cent of the males and 34.6 per cent of the females were infected with *Opalina ranarum* or *Cepedea dimidiata.*

B. fowleri: Of five living individuals from Massachusetts, 1 bore *Opalina obtrigonoidea;* of 12 living specimens from Raleigh, North Carolina, 2 bore many *Opalina obtrigonoidea.*

B. halophilus: Of numerous living specimens, nearly all were found to be infected with *Opalina obtrigonoidea maxima.*

B. marinus: Nearly all of about 12 living specimens bore *Zelleriella antilliensis.*

B. viridis: Of 4 living specimens from Naples, Italy, 1 bore *Protoopalina caudata;* 1 bore *Opalina ranarum,* and one yielded *Cepedea dimidiata;* one was uninfected.

Family RANIDAE.

Rana boylei: Numerous living specimens were examined. The notes are mislaid, but from memory I can say that about half were infected with *Opalina virguloidea.*

R. catesbeiana: 5 living specimens from North Carolina yielded no Opalinids; 7 half-grown living specimens from Massachusetts bore no Opalinids; 3 large living tadpoles from Massachusetts had no Opalinids; 11 full grown living individuals from Maryland bore no Opalinids; several living specimens from Ohio were uninfected. No Opalinids have been reported from this species, except that of 15 live specimens from the Hawaiian Islands (introduced) 2 bore *Cepedea dimidiata hawaiiensis.* It is said that Anura of several species have been introduced from Asia into Hawaii, and it seems probable that the American bullfrog, also introduced, adopted its Cepedeas from some Asiatic species, since none of these frogs in America have been found to harbor Opalinids. It is of interest, in this connection, to note that the southern bullfrog of America, *Rana grylio*, only preserved specimens of which have been available for study, has not been found bearing Opalinids.

R. clamitans: Of 24 living specimens from North Carolina none bore Opalinids; numerous living specimens from Ohio were found uninfected. In preserved specimens of this frog no Opalinids have been found. It is evident that this large, common American "green frog," like the still larger American bullfrogs of both species, rarely, if ever, carries Opalinids. Tadpoles, probably of this species, collected by Dr. Charles E. Simon, in Nova Scotia, bore *Opalinae* of a species tentatively named *O.* [*larvarum*], but we have no data as to the proportion of the larvae infected.

R. draytonii: Of numerous living specimens from near San Francisco, California, 1 held *Zelleriella ranaxena* and 2 carried *Opalina draytonii;* 4 other living specimens from south of San Francisco bore no Opalinids; 22 living specimens examined at Friday Harbor, Washington, bore no Opalinids, but they had been over a month in captivity. It seems that this species of frog is seldom infected.

R. esculenta: Of 77 living specimens examined by Metcalf, 59 carried *Cepedea dimidiata;* 2 or more bore both this species and its form *zelleri;* 16 bore no Opalinids. In reexamining some of this Bavarian material I find more than two, apparently six, specimens bore both forms of this *Cepedea*, the *zelleri* forms in four of the infections being not so large and stocky as in the two previously recorded. Andre (1913) reports that from 82 per cent to 89.2 per cent of frogs of this species from different localities in Switzerland are infected with *C. dimidiata.*

R. palustris: Of 3 living specimens from North Carolina, 1 bore abundant *Opalina obtrigonoidea* and 2 were uninfected; of 8 living specimens from Massachusetts, 4 bore *O. obtrigonoidea* and 4 were uninfected; of many speciment from Ohio, the author would say from memory that about one-third were infected with the same species of *Opalina.*

R. pipiens: Numerous living specimens from North Carolina, Ohio, and Illinois yielded *Opalina obtrigonoidea*, but the notebook containing the records is mislaid and from memory I can not say as to the percentage infected. Infections were not at all rare.

R. pipiens sphenocephala: Of 8 living specimens from North Carolina 2 bore *Opalina carolinensis.*

R. sylvatica: Of 5 living specimens from Ohio, 3 carried *Opalina virguloidea;* of numerous living specimens from North Carolina, about two-thirds bore the same *Opalina.*

R. temporaria: Of 15 living specimens examined by Metcalf, 10 bore *Opalina ranarum* and 5 had no Opalinids; of 233 specimens examined by Andre (1913) 173 bore *O. ranarum.*

These data are inadequate for any satisfactory answer to the question of the frequency of infection of the Anura by Opalinids. It seems clear that species differ in the proportion which are infected. Quite probably the American *Rana catesbeiana*, *R. grylio*, and *R. clamitans*, at least when adult, are never infected. Tadpoles, probably of *R. clamitans*, are sometimes infected. The European *Bombina pachypa*, on the other hand, is usually infected. The adults of the American *Hyla versicolor* seem to be infected in less proportion than are the tadpoles. Neresheimer's belief that all healthy frogs bear Opalinids is not borne out by a review of even our scanty data from living Anura. It is probably not true of any species of Anura.

Second.—The inquiry as to Anura which may be infected by more than one species of Opalinid is answered, so far as our data is concerned, by a glance at the following list.

Family DISCOGLOSSIDAE.

Bombina bombina: Protoopalina caudata, P. intestinalis, and *Opalina ranarum* (very rare).
B. orientalis: Protoopalina macrocaudata and *P. orientalis.*
B. pachypa: Protoopalina caudata and *P. intestinalis.*
Discoglossus pictus: Protoopalina "intestinalis" Opalina ranarum (Brumpt, 1915).

Family PELOBATIDAE.

Scaphiopus hammondii: Protoopalina hammondii and *Opalina oblanceolata.*
S. solitarius: Zelleriella scaphiopodos, Opalina oblanceolata, O. obtrigonoidea and *Cepedea* (?) "*flava.*"

Family HYLIDAE.

Hyla bandinii: Cepedea bandinii and *Opalina guatemalae.*
H. pickeringii: Opalina pickeringii and *O. obtrigonoidea.*
H. regilla: Opalina oregonensis and *O. virguloidea.*
H. septentrionalis: Zelleriella [of *Hyla septentrionalis*] and *Opalina* species unnamed.
H. versicolor: Opalina hylaxena and forms *orbiculata, parvinucleata* and *georgiana.*

Family BUFONIDAE.

B. bufo: Cepedea dimidiata, Opalina ranarum and *O. cincta.*
B. bufo asiaticus: Protoopalina axonucleata, Opalina bufoxena and *O. asiatica.*
B. cognatus: Opalina discophrya and *Zelleriella hirsuta.*
B. compactilis: Opalina gigantea and *O. spiralis.*
B. copei: Opalina discophrya and another unidentified but different *Opalina.*
B. fowleri: Opalina obtrigonoidea and form *rugosa.*
B. haematiticus: Cepedea dolichosoma. Opalina species (?) and *Zelleriella bufoxena.*
B. lentiginosus: Cepedea obovoidea and *Opalina triangulata.*
B. melanostictus: Cepedea formosae, C. pulchra javanensis, Protoopalina formosae and a doubtful record of *Zelleriella macronucleata.*
B. peltocephalus: Protoopalina bufonis and *Z.* sp. ?.
B. punctatus: Zelleriella [of *Bufo punctatus*] and *Opalina obtrigonoidea.*
B. regularis: Protoopalina regularis and *P. stevensoni.*
B. typhonius: Zelleriella opisthocarya subspecies (?) and *Opalina panamensis.*
B. viridis: Protoopalina caudata, Cepedea dimidiata and *Opalina ranarum.*
B. woodhousi: Zelleriella woodhousi and *Opalina woodhousi.*

Family LEPTODACTYLIDAE.

Limnodynastes dorsalis: Zelleriella binucleata, Protoopalina "intestinalis," P. dorsalis, and *P. acuta.*

Telmatobius jelskii: Zelleriella telmatobii and *Protoopalina longinucleata.*

Uperoleia marmorata: Protoopalina tenuis and *P. "intestinalis"* (Raff, 1911).

Family GASTROPHRYNIDAE.

Atelopus varius: Zeleriella [*atelopodos*] and *Z. atelopyxena.*

Rhinoderma darwinii: Zelleriella darwinii and *Protoopalina rhinodermatos.*

Family RANIDAE.

Polypedates leucomystax: Cepedea segmentata and, probably from this species, Dobell's *Opalina virgula.*

Rana draytonii: Zelleriella ranaxena and *Opalina draytonii.*

R. esculenta: Cepedea dimidiata and its form *zelleri, Protoopalina intestinalis* and *Opalina ranarum.*

R. limnocharis: Cepedea longa, Opalina lata and *O. japonica.*

R. nigromaculata: Protoopalina axonucleata lata and *Cepedea dimidiata orientalis.*

R. nutti: Protoopalina primordialis and *P. nutti.*

R. pipiens: Cepedea mexicana and *Opalina obtrigonoidea.*

R. temporaria: Opalina ranarum and its forms *arvalis* and *cinctoidea*, and *Cepedea dimidiata.*

R. temporaria parvipalmata: Opalina ranarum and its form *parvipalmatae.*

R. tigerina: Cepedea ophis and *Protoöpalina filiformis.*

Third.—Upon the question of the simultaneous infection of a single individual host by two or more species of Opalinids there is less data. Double or multiple infections by Opalinidae are not known from fishes or Urodela, from *Dendrobatinae*, or *Raninae.* Among the Discoglossidae, in *Bombina pachypa*, I found a single infection of distorted Protoopalinas in an individual with badly inflamed alimentary canal, in which there appeared to be both *P. caudata* and *P. intestinalis*, but the identification of these distorted parasites was very doubtful. Among the Pelobatidae, in an individual of *Scaphiopus solitarius*, was found *Zelleriella scaphiopodos* and *Opalina oblanceolata.* Among the Hylidae a *Hyla baudinii* gave *Cepedea baudinii* and *Opalina guatemalae*, and a *H. septentrionalis* yielded a *Zelleriella* of doubtful species and an unidentified *Opalina.* There was no doubt in this case, though the species of the Opalinids were not determined. Among the Bufonidae we have six cases of individuals simultaneously infected by two species of Opalinidae, as follows: *Bufo cognatus* bearing *Zelleriella hirsuta* and *Opalina discophrya; B. haematiticus* bearing *Cepedea dolichosoma* and an undetermined species of *Opalina; B. peltocephalus* bearing *Protoopalina bufonis* and *Zelleriella* sp. ?: *B. typhonius* bearing *Zelleriella opisthocarya* [of *Bufo typhonius*] and *Opalina panamanensis; B. woodhousi* bearing *Zelleriella* [of *Bufo woodhousi*] and *Opalina* *woodhousi.* Among the Leptodactylidae, in *Limnodynastes dorsalis* Raff reports *Protoopalina dorsalis* and *P. acuta* as present simultaneously; in *Uperollia marmorata* Raff reports simultaneous infection by *Protoopalina* "*intestinalis*" and *P. tenuis.* Among the Gastrophrynidae we have one instance, an individual of *Rhinoderma darwinii* showing *Zelleriella darwinii* and *Protoopalina rhinodermatos.* Among the Ranidae, in *Rana esculenta* we frequently find together *Cepedea dimidiata* and its form *zelleri.* Both Neresheimer and I have formerly treated the latter as a distant species.

No case of triple simultaneous infection is known. Of the eight known cases of double infection, two show both the Opalinids belonging to the same genus, while in six cases the two Opalinids are of different genera. In two of these six cases the Opalinids are of the same subfamily; in the other four cases they belong to different subfamilies. It is of some interest to note that five of the eight known cases of double infection are from species of *Bufo*, a genus which is hospitable to all genera and subgenera of Opalinidae.

9. A CHRONOLOGICAL REVIEW OF THE LITERATURE OF THE OPALINIDAE, SUPPLEMENTARY TO THE CHRONOLOGICAL REVIEW PUBLISHED IN THE YEAR 1909 AS A PART OF THE AUTHOR'S PAPER "OPALINA."

My former review of the literature of the Opalinidae included papers published as late as 1908. This section of the present paper will include references to a few books and papers omitted in the former former review and will bring the summary of the literature down to date.

Ehrenberg (1834) refers to *Bursaria* as including some species which have no mastication apparatus [Ehrenberg includes in the genus *Bursaria* probably three or four Opalinidae; *B. intestinalis* (= probably *Protoopalina intestinalis*, *P. caudata*, and *Cepedea dimidiata*) and *B. ranarum* (= *Opalina ranarum*)].

Pritchard (1842). The first edition of Pritchard's History of Infusorial Anïmalcules, published in 1838, did not mention any organisms which seem to be Opalinids. The second edition, four years later, lists among the Bursarias two forms which are probably Opalinidae, *B. intestinalis* [=*Protoopalina intestinalis*, *P. caudata* or *Cepedea dimidiata* or all three] and *B. ranarum* [= *Opalina ranarum*], though he describes each as having a small mouth. His *B. nucleus*, from the recta of *Rana temporaria* and *R. esculenta*, is probably *Balantidium* rather than an Opalinid. *Bursaria intestinalis* can not be "F. O." [O. F.] Müller's *Vibrio vermiculus*, as Pritchard thinks.

Pritchard (1852), third edition, places the genus *Opalina* in the "family" Opalinoea in the first order, Astoma, of the class Infusoria. The Astoma are defined as "Infusoria without mouth." The reference to *Bursaria* is repeated verbatim from the second edition, but he adds further discussion as follows: "The species called by Ehrenberg *Bursaria ranarum*, is the *Opalina ranarum* of those other authors [Purkinje and Valentin, Siebold, Dujardin]; or indeed this *Opalina* appears the representative of *B. ranarum*, *B. intestinalis*, *B. nucleus* [the latter probably not an Opalinid], and it may be also of other *Bursariae* of Ehrenberg, if, as some maintain, they are only varieties and not species. * * * The *Opalinae* are characterized by being oval or oblong [not true of *Protoopalina intestinalis* and *Cepedea dimidiata* known at that time as *Opalina intestinalis*], with an oblique cleft indicating a mouth toward the anterior extremity (Dujardin); though, according to Siebold, they have no mouth. They are parasitic chiefly in Frogs and Annelids, and form but an artificial and provisional genus, for if mouthless, they belong to the Parameciens; if they possess a mouth, to *Leucophrys*." [*Opalina lumbrici* and *O. naidum*, the two species described, are neither of them true Opalinids. Of course the references to the mouth are inadvertently reversed.] The references to Opalinidae, given in the fourth edition (1861) of Pritchard's History of Infusorial Animalcules, are noted in Metcalf (1909).

Stein (1854) denies that *Opalina ranarum* is an independent organism, rather than a development stage of some higher animal, because it has no mouth, a point which he discusses (p. 181), mentioning Ehrenberg's (1838) [mistaken] reference to the presence of a mouth. He says *Opalina ranarum* shows no trace of the characteristic Ciliata nucleus and reports that he never saw reproduction by fission, but mentions observing a single one out of many hundred individuals which was constricted, but in so unusual a direction that it could be called only an injured animal. [Quite likely this was the so-called oblique division, really morphologically longitudinal. This is the earliest reference I have found to what may be fission in *O. ranarum*, but Ehrenberg (1838) had described transverse division in "*Bursaria*" *intestinalis*, = *Protoopalina intestinalis*, *P. caudata* or *Cepedea dimidiata* or probably all three. Ehrenberg's references do not enable us to distinguish these three forms from one another.] He also mentions the absence in *Opalina ranarum* of any organs for attaching to the alimentary canal wall of the host.

Bronn (1859) does not include *Opalina* among the Protozoa, but in a footnote (p. 124) writes "Die wirklich Mund-lose Sippe Opalina PV (=Leucophrys Duj.. nicht Ehrb.), welche parasitisch in anderen Wasser-Thieren lebt, besteht nach Stein's Nachweisungen aus Entwicklungs-Zuständen, theils von anderen Infusorien (*O planarium* von *Trichodina mitra*), theils vielleicht von Binnen-Würmern (Distomen?). While there is here no mention of true Opalinids, the implication seems to be that Bronn would regard them in the same way, as developmental forms of other organisms, an opinion already expressed by Max Schultze (1851) as well as Stein (1854, 1856).

Hoffer (1872, or later) quotes from Leydig the statement that *Opalina ranarum* is multinucleated, but the true nuclear nature of the bodies is questioned. The opinion is expressed that, if they be true nuclei, Kölliker's and Schultze's interpretation of the Opalinas as developmental stages of higher organisms is more probable. *Opalina dimidiata* and *O. obtrigona* also are mentioned. Under Ciliata Holotricha he places "I Fam. Opalinina (wahrscheinlich zum grössten Theile keine Infusorien)" including the genera *Opalina*, *Hoplitophrya*, *Anoplophrya*, *Haptophrya*.

Kent (1880). Reference is made to the Opaninidae, including the astomatous Ciliata, among them being *Opalina*, "the simplest of them all." The contractile vacuole is said to be absent; the nuclei are recognized, and the growth from the uninucleate condition of "young forms" to the multinucleate "adult" is mentioned. There are five figures of *O. ranarum*, "adult," cyst, transverse [?] fission, and what appears to be a microgamete mother-cell. [The latter must have been from a tadpole, if it be a mother-cell.]

Balbiani (1881) mentions absence of mouth and anus in "*Opalina*," limiting the genus to the forms parasitic in Batrachians.

Kent (1881-1882). In the reference to this book, in the author's previous review of the literature, Metcalf (1909), it would have been well to have quoted Kent's classification of *Opalina*, as follows:

Appendix A.—Holotricha-Astomata.

Fam. XIII, Opalinidea; animalcula finely and evenly ciliate throughout; endoparasitic, possessing no distinct oral aperture	Simply ciliate, possessing no special prehensile organs	Contractile vesicle absent, endoplast rarely conspicuous	"*Opalina*"
		One or more contractile vacuoles, endoplast conspicuously developed	*Anoplophrya*
	Provided with supplementary prehensile organs	Prehensile organs acetabuliform	*Haptophrya*
		Prehensile organs unciform	*Hoplitophrya*

Kent's use of the name *Opalina* covers only the four present genera *Protoopalina*, *Zelleriella*, *Cepedea*, and *Opalina*.

Blochmann (1886) refers to the members of the genus *Opalina* as lacking mouth, anus, and contractile vacuole, as possessing numerous regularly distributed small nuclei, and as occurring in the recta of native [German] frogs and toads. No figures.

Gruber (1886) describes as the common method of reproduction in Opalinas the fragmentation of the body into small pieces and the subsequent growth of these pieces to normal animals. He says it is noteworthy that these very organisms can not be artificially multiplied, but that this is probably due to the fact that they are endoparasites and that it is difficult to furnish them natural environmental conditions in artificial cultivation.

Fabre-Domergue (1887) refers to Bütschli's (1887–1889) studies of protoplasmic structure in *Opalina*.

Stokes (1888) describes and figures *Opalina* [*Cepedea?*] *flava*. The description is insufficient for the recognition of the species.

Fabre-Domergue (1890) refers to the absence of contractile vacuole in *Opalina ranarum*.

Shewiakoff (1886) gives full reference to *Opalina ranarum* (figure, *O. obtrigona*, *O* [*Cepedea*] *dimidiata* (figure), *O.* [*Cepedea?*] *flava*, *O.* [*Protoopalina*] *intestinalis* and *O.* [*Protoopalina*] *caudata* (figure). Cilia and cytoplasm are described; mouth, anus, and contractile vacuole are said to be lacking. "The macronucleus [!] consists, in a majority of species, of many discoidal [spherical, ellipsoidal or pear-shaped, not discoidal], round nuclei, scattered without any order through all the endoplasm. Each nucleus contains small, highly refractive nucleoli [one true nucleolus in each nucleus. The reference here may be to the macrochromatin masses]. In two species (*intestinalis* and *caudata*) there are in addition to many nuclei [he must mean here the endospherules] one macronucleus consisting of two spherical or oval portions united by a fine thread [this must mean the two nuclei]. Apparently there is no micronucleus. Movements not especially quick, body very supple, not contractile, nutrition by endosmosis over whole surface of body, transverse and oblique fission ("very probably the so-called oblique division is nothing less than conjugation"), encystment, several nuclei in the cysts at first, after they have lain in water a time the cysts have but one nucleus [erroneous] so that the young individuals hatched from the cysts have but one nucleus. [The number varies from 1 to 12 or so; they have the same number with which they entered the cysts.] There are literature and synonomy lists for each of the six species described. *Cepedea dimidiata*, *Protoopalina intestinalis* and *P. caudata* are mistakenly described as a little flattened. There is an extended discussion of the history of the taxonomy of the Ciliata in which the classification of the Opalinids by different students is given. Some of those whose classifications of Opalinidae are mentioned are Ehrenberg (1831 and 1838), Dujardin (1841), Perty (1852), Stein (1859), Claparede and Lachmann (1868), Kent (1881–1882), Bütschli (1880–1889, Infusorian part 1887–1889).

Entz (1888) mentions several systems of classification, by different authors, including the Opalinids: he refers to Engelmann's (1876) opinion that the nucleuslike structures in *Opalina ranarum* are true nuclei, but that *O. ranarum*, like other multinucleate ciliates, is still unicellular. The rodlike bodies may be trichocysts as Allman and most observers believe, or tactile organs (Tästkörperchen) as Stein thought [apparently they are nutritive plastids]. Opalinids on a microscope slide are disturbed by and retract from strong light [an observation not confirmed, so far as the author of this volume knows]. Contractile vacuole wanting in most Opalinids [the exceptions, in his meaning, being, of course, of genera not to-day classed as Opalinidae]. No mouth or anus or digestive chamber. No reserve nucleus (Reservekern) [micronucleus (?), not true of some of the genera then classed with, but now removed from the Opalinidae]. Nuclei of multinucleated species passive during body division. Fission transverse, oblique or longitudinal. Zeller's studies upon reproduction and life history are described rather fully.

Mengarini (1896) says that in *Opalina* [species?] from *Rana* [species?] the pellicle serves as an osmotic membrane. Observations and experiments are reported.

Tönniges (1898). In the author's previous reference to this paper (Metcalf, 1909) mention was omitted of Tönniges' definite denial of the interpretation of longitudinal fission as conjugation, as some had claimed, and of any occurrence of conjugation during the multinucleated stage of the life cycle. Tönniges says that during encystment the several nuclei fuse "unter sehr bemerkenswerthen Erscheinungen" [not described], so that the individuals which hatch from the cysts in the tadpole are uninucleated [not confirmed by my observations], and these uninucleated individuals conjugate within the tadpole alimentary canal [no description of the process or of the appearance of the gametes or zygotes]. Succeeding conjugation, there begins active multiplication by longitudinal and transverse fission and by budding. Transverse division is described fully. The products of such division are sometimes unequal in size. When the disparity is great the process may be termed budding. Longitudinal and multiple division are also described. The divisions of the nuclei have no constant relation to the divisions of the body, and from this fact the conclusion is drawn that the divisions of the nuclei are automatic and without control from the cell body. [Upon this point see Hegner and Hsiang-Fong Wu (1921).]

Schweier (1900) gives an identification table for the species *ranarum*, *obtrigona*, *dimidiata*, *flava*, *intestinalis*, and *caudata*, with descriptions, figures, literature lists, and synonymy for each species.

Entz (1901 and 1904) says that *Opalina ranarum*, through most of the year, shows similar size, division being infrequent. The last of April or the first of May occurs a mania for division, both transverse and "oblique," until 1 or 2 nuclei [Metcalf, 1 to 12] are present in each small individual, when encystment occurs. The cysts pass with the feces of the host into the water and are taken with the food into the tadpoles. Here the cysts hatch out and the little Opalinas rapidly develop into multinucleated individuals of normal size.

Shipley and McBride (1901) make brief reference to Opalinidae. Their statements in regard to division are inaccurate: "When division commences it continues until the resulting pieces have only one nucleus each; they then grow and do not divide again until they acquire the size they had before division took place and also the same number of nuclei."

Sand (1901–1902). This paper is not obtainable in this country. A brief reference to it suggests that it may discuss in part the reactions of Opalinids to certain poisons.

Enriquez (1902) regards the phenomena of ingress of food and egress of water in *Opalina ranarum* as physiological and not purely osmotic.

Zahn (1902) reports experiments upon *Opalina ranarum*, using 1 per cent solutions of various poisons in spring water, observing form, vacuolization and locomotion, after 5, 15, 25 and 35 minutes. The Opalinas were completely destroyed by Fluornatrium after 35 minutes, by Hydroxylamin after 25 minutes, by Formaldehyd after 5 minutes. Three nerve poisons caused only functional disturbance. With Curari, 0.001 per cent solution after 4 hours, "vollständige Lähmung herbeiführte." Conclusion: The substances named above, and also strychnine and morphine, are protoplasmic poisons, though cessation of motion and ultimate death in an Infusorian after treatment with chemical substances may be due not to direct protoplasmic poisoning, but to interference with its functions, leading to degeneration.

Keble and Gamble (1903) refers to the results of Conte and Vaney (1902).

Hickson (1903). The reference to Hickson's statements in my previous review of the literature (Metcalf, 1909) was confused. Beginning with the last sentence on page 140 it should have read, quoting from Hickson: "The mitotic figures described by Pfitzner are clearly seen in a large number of sections examined, but they are smaller than the meganuclei [no] and are probably formed by micronuclei which, as in other forms, increase considerably in size before division." [Each nucleus of *Opalina* seems to be functionally comparable to both micro- and macronucleus of higher Ciliata, but to be homologous with each, both the nuclei of Ciliata being, both phylogenitically and ontogenetically, complete nuclei.]

Kofoid (1903) places "*Opalina*" with *Protophrya*, *Anoplophrya*, *Hoplitophrya*, *Discophrya*, and *Opalinopsis*, in the family Opalinidae, giving as diagnostic description of "*Opalina*" [which included, of course, the four present genera] "body flattened [not in

Protoopalina and *Cepedea*], asymmetrical, many round nuclei or a single bipartatite nucleus" [often two nuclei, though none of the flat species known at the time of this paper had two nuclei or a double nucleus; nuclei frequently not round.]

Kunstler (1903) discusses and figures the pellicle of *Opalina* [*Cepedea*] *dimidiata*.

Birukoff (1904) says that under stimulation by direct electric current "*Opalina*" in sodium chloride solution moves to the anode; under stimulation by alternating electric current the *Opalinae* lie near the electrode, the *Paramecia* in a line outside the *Opalinae*.

Entz (1904), same paper as Entz (1901).

Gineste (1905) discribes again observations by Kunstler and himself upon the structure of the protoplasm in "*Opalina*" [*Cepedea*] *dimidiata*. [See Claoue (1919), page 445 of this paper, also see Metcalf (1909) for comments upon these often published results of Kunstler's and Gineste's studies.]

Birukoff (1906) briefly discusses the work of Wallengren and Statkewitsch upon galvanotaxis in *Opalina*. *Opalina* in physiological salt solution, under stimulation, invariably moves to the anode, a reaction the reverse of that seen in *Paramecium*. The difference in behavior may be due to the anterior end of *Paramecium* being more sensitive, while the two ends of *Opalina* are equally sensitive. In an acid medium *Opalina* moves to the cathode. The sensitiveness of *Opalina* may be increased in an acid medium. "Die Übersicht der neuesten Arbeiten über die Galvanotaxis führt zur unumgänglichen Schlussfolgerung, dass die allgemeine Erregbarkeit und kataphorischen Stromwirkung (Stromingsströme) in Wirklichkeit die beiden Hauptfaktoren der Galvanotropismus sind."

Schouteden (1906) reviews Shewiakoff's (1896) paper.

Mengarini, Margherita Scala, and Alberto Scala (1906) report observations upon the effect of sodium chloride solutions upon "*Opalina*," producing change of form, the permeability being different at the two ends of the body.

Galli-Valerio (1907) reports the presence of *Opalina ranarum* in *Rana esculenta* [new host record], *R. temporaria*, *Bombina bombina* [new host record] and *Triturus alpetris* [new host record], all from the Swiss canton Vaud.

Klopstocz (1907) gives a brief report of Neresheimer's (1907) work.

Mengarini and Scala (1907) report investigation of the action upon "*Opalina*" of solutions of sodium carbonate, sodium chloride, and potassium carbonate. Many experiments, fully described, statements being made as to the time the animals live in the different solutions, as to the extrusion of posterior globules, and as to color reactions.

Schouteden (1907) copies Bezzenberger's (1904) table for identification of Opalinidae and gives synonomy list and habitat for each species, *ranarum*, *lata*, *obtrigona*, *dimidiata*, *coracoidea*, *longa*, *flava*, *lanceolata*, *macronucleata*, *intestinalis*, and *caudata*.

Lebedew (1908) compares nuclear conditions in *Trachelocerca* and "*Opalina*," also their reproduction.

Dobell (1909) says: "It is certain, as Neresheimer hinted from his study of *Opalina*, that the parasites from Cephalopoda are not related to *Opalina* but to *Anoplophrya*."

Galli-Valerio (1909) mentions *Opalina ranarum* as occurring in *Bufo bufo* from Sondrio, Italy.

Hartmann (1909) refers to Nersheimer's (1907) description of reproductive chromidia in *Opalina ranarum* and to Metcalf's (1909) opposed results from study of binucleated species, and also refers to Metcalf's tentative suggestion of the origin of the endospherules from chromidia.

Metcalf (1909) describes in detail the structure, mitosis, and life history of *Protoopalina intestinalis* and *P. caudata;* describes the structure of *Cepedea dimidiata* and gives some data as to its life history; describes the structure of *Opalina obtrigona*, some phenomena of its nuclear degeneration, and its irregular presexual fissions; describes the structure of *Opalina ranarum* and gives some data as to its life history. Data are given as to infections found in nature and many experiments in infecting Anura with cysts of unaccustomed species of Opalinids are described; also the reactions of different portions of the body and its contents to many intra-vitam stains. There are included discussions of the evolution of mitosis, of the nature of the plastids of Opalinids, of the phylogeny of the nuclei of the Ciliata, of chromidia, of the phenomena of reduction, and of the relationships of the Opalinidae. Abnormal conditions are described in *Opalina obtrigona*, *Protoopalina intestinalis* and *P. caudata*. There is a description of *Cepedea dimidiata* form *zelleri* ["*Opalina zelleri*"] and a chronological review of the literature of the Opalinidae (*sensu stricto*) from Leeuwenhoek in 1865 to Löwenthal in 1908.

Dobell (1910) reports an unidentified, small, multinucleated "*Opalina*" from *Rana tigerina* and describes a new species, *Opalina virgula*, *from* "*Rhyncophorus maculatus*" [*Polypedates leucomystax?*], all from Ceylon.

Von Linden (1910) describes remarkable, irregular protuberances ("tentacles") from the body of *Cepedea dimidiata*. These are long and thin, irregularly arranged and of different numbers (2, 4, 5, 6) in different individuals, "usually 5." Both ectosarc and endosarc (with endospherules) are present in these protuberances, which are ciliated like the body. In occasional examples a protuberance is dichotomously branched. After 18 hours in salt solution the appearance is unchanged. The protuberances are said to be formed by a split appearing in the body, the tip of the protuberance being the last portion, except the persistently attached base, to separate from the body. Von Linden discusses the question whether these are atypical locomotor organs, or are pathological, or are connected with division, inclining to the latter interpretation, though complete separation was not observed. [These phenomena seem to be similar to, though more exaggerated than, those Metcalf (1909) described for *Opalina obtrigona* when the presexual mania for fission begins.] Von Linden's figures look much more like *Opalina ranarum* than *Cepedea dimidiata*. The arrangement of the nuclei in longitudinal rows (see his figures 1 and 2) is different from anything I have observed in any *Cepedea* or *Opalina*.

Cepede (1910) gives full reference to the discussions of the taxonomy of the true Opalinidae and the other Ciliata Holotricha Astomata. There is no detailed treatment of the true Opalinidae in Cepede's own discussion. This fine paper very clearly shows that the true Opalinidae are not closely related to the other astomatous Ciliates.

Cleland and Johnston (1910), "*Opalina* sp." This degenerate ciliate infusorian is comparatively common in the intestine of *Hyla aurea*, *H. caerulea*, *Limnodynastes peronii* and *L. dorsalis*. [Of course these parasites were not true *Opalinae* according to our present taxonomy but probably *Protoopalina*. The record from *Hyla caerulea* is new.]

Faure-Fremiet (1910) refers to paramyelin in "*Opalina*" as a product of degeneration of the cytoplasm, a combination of soluble and insoluble albuminoids and certain molecules of fatty acid; refers to the work of Balbiani, Kunstler and Gineste, Gineste, and especially Metcalf, upon cytoplasmic spherules.

Hartman (1910) compares the ectoplasm of *Trichonympha* "*hertwigi*" with that of "*Opalina*," also discusses gamete formation in the two genera. He rejects the idea of genetic relationship.

Nägler (1910) refers to Schouteden (1907).

Cockerell (1911) mentions "*Opalina* sp." as occurring in "the frog" in Boulder County, Colorado, United States of America.

Doflein (1911), in this fourth edition of his textbook, gives a brief description of the structure and life history of *Opalina ranarum*. Brief reference is made to the cilia, to the presence of true [?] glycogen and fat and to the excretory organs. The Opalinidae are classed primitive members of the Ciliata Holotricha. The ectoplasm is erroneously stated to be homogeneous, while the endoplasm is granulated. Metcalf is mistakenly quoted as saying that *Opalina ranarum* has a single, canal-like, excretory apparatus. [Some species of Opalinidae do have, but not *O. ranarum.*] The [morphologically longitudinal] fission is referred to as diagonal.

Raff (1911) describes and figures the new species *Zelleriella* ("*Opalina*") *binucleata*, from *Limnodysastes dorsalis* and *L. tasmaniensis*, and *Protoopalina* ("*Opalina*") *hylarum*, from *Hyla-aurea. Protoopalina intestinalis* [?] is mentioned as present in abundance in *Hyla aurea*, *H. ewingii*, and *Limnodynastes dorsalis.*

Stevenson (1911) describes an "Opalina-like form, which I am naming in this paper *Protoopalina stevensoni.* See p. 50.

Andre (1912, *a*). This paper I have been unable to obtain in this country.

Andre (1912, *b*) reports that in *Bufo bufo* ("*vulgaris*") *Opalina ranarum* and possibly also *Cepedea* ("*Opalina*") *diminiata* are present, along with other protozoan parasites, in the intestine, there being no incompatability between the different protozoan parasites. There are different proportions of infection of *Bufo bufo* in different localities. Of males 48.7 present were found infected, of females 34.6 present.

Hall (1912) mentions "Opalina" [species ?] collected at Ramaley, Boulder County, Colorado, reported by Cockerell in 1911.

Hartmann (1912) mentions absence of macronuclei in Opalinids; their "anisogamous merogamy (Metcalf, 1909) like that of Rhizopods and Sporozoa; and their" binuclearity [pseudo pleurinuclearity would be more accurate].

Metcalf (1912) describes *Protoopalina* ("*Opalina*") *mitotica* from *Ambystoma tigrinum* from Lincoln, Nebraska, a new species whose resting nuclei are in a metaphase condition of mitosis. The origin of the binucleated condition of the Ciliata is discussed. [*Protoopalina mitotica* is the first organism, animal or plant, reported whose resting nuclei are in a mid-mitotic condition.]

Raff (1912) searched the Australian forms *Hyla lesuerii*, *Heleioporus pictus*, *Pseudophryne semimarmorata*, *P. bibronii*, *Crinia signifera*, *C. frogatti*, and "*Hyperolia*" [*Uperoleia*] *marmorata* for Opalinids and describes the following new species; giving them all, of course, the genus name *Opalina*; *Protoopalina tenuis* from *Crinia signifera* and *Uperoleia marmorata*, *P. dorsalis*, and *P. acuta* from *Limnodynastes dorsalis.* She reports P. "intestinalis" from *Uperoleia marmorata.* "Abnormal forms" of *Zelleriella binucleata* (?), with from two to eight nuclei, are described from a specimen of *Limnodynastes dorsalis.*

Swarzewsky (1912) refers to Neresheimer's (1907) account of the formation of generative chromidia which give rise to gametic nuclei, also to Metcalf's (1909) opposed account according to which the gametic nuclei are direct derivatives of the ordinary nuclei, vegetative chromidia being formed previous to sexual reproduction. He says chromidia are formed during only part of the life cycle. These are vegetative chromidia (and possibly also reproductive chromidia, Neresheimer), function unknown. He says Hartmann considers the chromidia vegetative (after Metcalf). Swarzewsky quotes Neresheimer's observations of reproductive chromidia and says they can not be accepted without further investigation.

Andre (1913), notes the occurrence of *Opalina ranarum* and *Cepedea* ("*Opalina*") *dimidiata* in both *Rana temporaria* and *R. esculenta* [The latter is an unusual host record for *O. ranarum*], 173 out of 233 specimens of *R. temporaria* being infected, the males and females equally so. Opalinids and Flagellates are present in inverse proportions, that is, if the Opalinids are abundant there are few Flaggellates and *vice versa*. *Rana esculenta* is chiefly infected with *Cepedea dimidiata*, though some have *Opalina ranarum*. One frog of this species was found bearing *Protoopalina* ("*Opalina*") *intestinalis*. In this frog *Cepedea dimidiata* lives less readily with the Flagellates. He says 82 to 89 per cent of *Rana esculenta* in different localities are infected with Opalinids.

Awerinzew (1913) describes the new species *Protoopalina* ("*Opalina*") *primordialis* from *Rana nutti* from German East Africa. Awerinzew is quoted in full in the present paper, Section 3, under *P. primordialis* on page 31.

Collin (1913) describes the new species *Opalina cincta* from *Bufo bufo* ("*vulgaris*"). He reports *Protoopalina* ("*Opalina*") "*intestinalis*" from *Pelobates cultripes* from Montpellier, France. He notes that both *Nyototherus* and "*Opalina*" in the spring infect tadpoles of Anura by means of cysts, a similarity of habit doubtless due to parallel adaptation to similar conditions of parasitism. He reports cysts of Oplinids in tadpoles of *Alytes* and *Bufo calamita*.

Lühe (1913). I have failed to find in this country the 1913 edition of Lang's Lehrbuch which contains Lühe's discussion.

Poche (1913) refers to the uniformity of the nuclei in "*Opalina*." He gives a classification, as follows:

VII Classe, INFUSORIA.

I Ordnung, Holotricha.
I Unterordnung, Opalininea.
I Familie, Opalinidae.

One genus, only, "*Opalina*" [including, of course, the four present genera]. Hartog's (1906) and Neresheimer's (1907) opinions that Opalinids should be classed among the Flagellata are rejected in favor of Metcalf's (1909) view that they are related to the Infusoria Holotricha.

Collin (1914) compares *Opalinopsis carinaria* with "*Opalina*" [*Protoopalina*] *saturnalis.*

Metcalf (1914) describes "*Opalina*" (*Zelleriella*) *antilliensis*, from *Bufo marinus* from Jamaica, West Indies (introduced), and especially its double set of chromosomes; that is, 10 macrochromosomes, metabolic, and 10 microchromosomes, reproductive. Comparison is made of its mitosis with that of an unnamed species from *Bufo halophilus*, which in the present paper is described and named *Opalina obtrigonoidea maxima.*

Brumpt (1915) reviews the work of Neresheimer (1907) and of Metcalf (1909) upon the life history of the Opalinids and adds important comments and new data from his own studies, which we must report at some length. He confirms Metcalf against Neresheimer, saying that the Opalinids have microgametes, and macrogametes; Brumpt studied the phenomena of fertilization in *Protoopalina intestinalis* from tadpoles of *Bufo mauritanicus*, in *Opalina obtrigona* from tadpoles of *Hyla orborea*, in *Cepedea dimidiata* from tadpoles of *Rana esculenta*, and in *Opalina ranarum* from tadpoles of *Rana temporaria;* he observed the gametes of *Protoopalina intestinalis* from tadpoles of *Bombina pachypa* and of *Bufo calamita:* and he studied the phenomena of fertilization in unidentified species of Opalinids from tadpoles of *Bufo bufo* and of *Discoglossus pictus.* In all these forms the phenomena were identical. He placed cysts of Opalinids in water for 24 hours or more before using them in experimental infections. The unencysted Opalinids died, leaving only the cysts alive. The Opalinids, emerging after some hours from the ingested cysts when these have reached the recta of the tadpoles, rapidly divide (3 to 10 longitudinal divisions) before the gametes are formed. Toward the 36th hour macrogametocytes begin to appear, also microgametes and paired couples. The number of these elements increases for several days, but about the tenth day microgametes and copulating pairs are seldom found.

Metcalf is confirmed in the statement that the microgametes are always uninucleate, while the macrogametes are sometimes binucleate. Neresheimer described encystment of the zygotes soon after conjugation. Metcalf did not observe this. Brumpt found cysts in tadpoles of numerous species, but not immediately after fertilization. He says the zygates divide repeatedly after fertilization. When the rectum of the tadpole has become filled with these individuals, which have the same shape as the forms in adult Anura, there comes on after some weeks an epidemic of division followed by encystment, paralleling the presexual phenomena among the Opalinids in the adult Anura in the spring of the year. Not all the Opalinids encyst and go out of the tadpoles; some remain in the tadpole and continue its infection [as is the case with the adult Anura in the spring]. Cysts were found in tadpoles of *Bufo bufo* after 16 days; and of *Discoglossus pictus* after one month; of *Rana esculenta* after 6 weeks; of *Hyla arborea*, naturally infected, which were about a month old. Tadpoles of *Rana temporaria* and *Bufo bufo*, about two months old and ready to metamorphose, almost always showed some cysts. All of these cysts are identical morphologically (biologically ?) with those found in the adult Batrachians [but are different from the " copulation cysts with large, spindle-shaped nuclei " described by Neresheimer]. Brumpt has not seen cysts containing more than one Opalinid, though Leger and Duboscq and also Löwenthal have described them [I have seen one cyst containing an individual which was in process of division, probably having been in fission when encystment occurred].

Cysts of Opalinids from tadpoles, ingested by young tadpoles of several species develop in every way typically. They form gametes after 24 to 48 hours and their further development is normal. After about three weeks, in one instance, new infection cysts were formed in the tadpoles. The following infections were made: Young tadpoles of *Bufo bufo* with cysts from tadpoles of *Rana temporaria;* young tadpoles of *Discoglossus pictus* with cysts from tadpoles of *Hyla arborea;* young tadpoles of *Discoglossus pictus* with cysts of *Protoopalina intestinalis* from tadpoles of *Discoglossus pictus.* In the last case, after three weeks, the tadpoles ejected the cysts.

If infected tadpoles, in which the Opalinids have gone through the sexual process, are reinfected with more cysts, the later cysts hatch, but their Opalinids after emergence do not go through the sexual process, but apparently develop directly to adults. But previously uninfected tadpoles, newly infected from the same lot of cysts used in the preceding experiment, do show gametes after 48 hours.

Young tadpoles of numerous species infect themselves with cysts of diverse species of Opalinids. When a tadpole is already infected with its accustomed species of Opalinid, it is resistant to further infection. How is it that one species opposes itself to the adoption of a second species which is able to develop when the first species is absent? We may believe that there is some vital interaction between parasite and host which varies with the species of host. Two species (*Protoopalina intestinalis*, *Opalina ranarum*), which do not seem able to live together in tadpoles of *Rana esculenta* and *R. temporaria*, live very well in tadpoles of *Discoglossus pictus* whose adults in a state of nature show both of these species of Opalinids.

Cross infections (with unaccustomed species of Opalinids) are of comparatively short duration, as are also infections of tadpoles by adult Opalinids. To produce a permanent infection tadpoles must ingest cysts. Brumpt regards *Protoopalina caudata* as a variety of *P. intestalinis* [the two forms seems to me better treated as distinct species].

Metcalf (1918,*a*) read before the American Society of Zoologists a paper, an abstract of which was published, as follows: "*Opalina and the origin of the Ciliata.* Opalina characters, 1, reproduces by both longitudinal (Flagellata) and transverse (Ciliata) fission; 2, sexual act complete fusion of dissimilar gametes (Flagellata); 3, no kinetic center (centrosome [unique]); 4, kinetoplasm in form of basal granules of the cilia and a network of neural fibrillae connecting these (Ciliata); 5, uninucleate at sexual period, pleurinucleate during the rest of the life cycle [unique]; 6, in pleurinucleate condition all nuclei alike; 7, in each nucleus trophochromatin and idiochromatin distinct (except in origin); in the binucleate species the trophochromatin is in massive chromosomes of constant, definite form and number, apparently equal in number to the granular idiochromosomes, and their division in mitosis is regular; in the multinucleate species the trophochromatin masses are not constant in number. size, or form, and may divide irregularly in mitosis; the trophochromatin is extruded from the nuclei at the sexual period; 8, the pseudopleurinucleate condition is due to temporary suppression of the divisions of the body, the nuclei having divided; 9, this delay in completion of mitosis affects also the nuclei, which in numerous species do not complete their division promptly, but come to "rest" in different stages of the incomplete mitosis. In the 60 (?) species studied, 40 (?) of them new [this now proves to be 152± and 137±], a complete series is seen from uninucleate forms with single nucleus in an anaphase of mitosis, through uninucleate [*i. e.*, with two daughter nuclei still connected] species with telophase nuclei. binucleate species with resting nuclei of the usual type, binucleate species with two prophase nuclei, still others with two telophase nuclei. quadrinucleate species, multinucleate species, and finally an elongated multinucleated species whose transverse body divisions have started but are arrested while incomplete, giving an appearance of metamerization. The binucleate Opalinids form a genus. *Protoopalina* [now divided into two genera, *Protoopalina* and *Zelleriella*], distinct from the multinucleate species, *Opalina* proper [now *Cepedea*+*Opalina*], the chief distinctions being in nuclear characters. The Opalinidae are an offshoot from the primitive Ciliata before the latter had acquired true binuclearity and the subsequent dimorphism of nuclei. They should be classed as Protociliata, under the Ciliata. The Astomata [other "Astomata"] (*Discophrya*, *Anoplophrya*, *Hoplitophyra*, etc. ?) are Euciliata, having arisen later, after the dimorphic nuclei were acquired. They are not closely related to the Opalinidae?

Metcalf (1918, *b*) gives a brief abstract of a lecture before the Biological Society of Washington. The lecture described, and the abstract made reference to, the series of mitotic conditions observed in the "resting" nuclei of different species of Opalinids, and the origin of the binucleated condition in the Ciliata was discussed. A classification of the Ciliata was given, as follows:

- Ciliata.
    - Protociliata.
        - Opalinidae.
            - *Protoopalina.*
            - *Opalina.*
    - Euciliata.

Pinto (1918) describes and adequately figures the new species "*Opalina*" (*Zelleriella*) *brasiliensis* from *Leptodactylus ocellatus.*

Cordero (1919) reports Opalinids, which he identifies with *Zelleriella antilliensis*, from *Leptodactylus ocellatus* from La Plata, *Leptodactylus prognathus* from Tacuarembo, Uruguay, and from *Bufo arenarum* from Montevideo. [It seems possible that more detailed study of the parasites in these three species would show them not to be of the species *antilliensis.*] Twelve out of fifteen specimens of *Bufo arenarum* were infected. Only one infected individual each of *Leptodactylus ocellatus* and of *L. prognathus* were found. Six specimens of *Hyla* "*raddiana*" [*pulchella*] were without Opalinids. *Nyctotherus* (*cordiformis*?) was associated with *Zelleriella* in *Bufo arenarum* and *Leptodactylus ocellatus. Zelleriella antilliensis*, or a closely related binucleated species, was found in *Pseudis mantidactyla*, *Paludicola bibronii*, *Limnomedusa macroglossa* and *Bufo d'orbignyi* ("*orbignyi*"). [The identification of these forms with the species *antilliensis* should be reviewed.]

Claoue (1919) reports again Kunstler's observations and views as to the protoplasmic structure in *Cepedea dimidiata.*

Metcalf (1920,*a*) discusses the classification of the Opalinidae, summarizing it as in the present paper (see p. 271).

Metcalf (1920,*b*) discusses the occurrence of Leptodactylidae infected with *Zelleriella* in both South America and Australia and emphasizes the conclusiveness of the evidence from the distribution of the hosts with their parasites that South America and Australia were once connected by land. He discusses also, from similar data of distribution of Anura and their Opalinid parasites, the early separation of Patagonia from tropical America and their later union, and urges the use of such study of double or multiple series of data from hosts and their parasites in investigating problems connected with geographical distribution.

Honigmann (1921). A notice [not quite accurate] of Metcalf 1920, *b*) showing from the Anura and their Opalinid parasites the importance of the evidence from such a double series of data bearing upon questions of former intercontinental connections.

Metcalf (1921,*a*) gives an abstract of a paper read before the American Society of Zoölogists, as follows:

Geographical distribution of the Anura and their Opalinid parasites. The Amphibia give peculiarly valuable evidence as to land migration routes, for they can not endure salt or even brackish water. The Opalinids are parasitic exclusively in Anura, excepting three species, two of which are known from Urodeles and one, strangely, from a marine fish. The author has studied 130 species and 20 subspecies of Opalinids from all temperate and tropical portions of the world, southern Asia being the only region outside the northern and southern cold belts from which his material is not quite abundant. Study of this material and comparison with the known data as to the geographical distribution of the Anura indicates: 1, That the *Raninae* are a northern and eastern hemisphere subfamily, which have not reached Australasia (except for a single species at the northern tip of Australia), and have not reached South America (except for three species which have entered the northern Andes): 2, That the Bufos arose in the northern hemisphere [the present volume places the origin of *Bufo* probably in northwestern South America or possibly in southeastern Asia] and were not present in Antarctica, Patagonia, and Australia at the time when these were united into an Antarctic continent, though the Bufos are now in Patagonia: 3. That the Leptodactylidae arose in Patagonia, at a time when Patagonia was not connected with tropical America, and spread *via* Antarctica to Australia: 4, That later than this, Patagonia having separated from Antarctica and having united with tropical America, the Bufos passed southward into Patagonia and the Leptodactylids passed northward into tropical America. When the Bufos and Leptodactylids met, the Bufos adopted the Leptodactylid Opalinids, while the Leptodactylids did not adopt the multinucleated Opalinids of the Bufos: 5, That the Hylidae arose comparatively late in the Antarctic continent and did not reach tropical America until Patagonia united with tropical America: That having reached the tropical forests of America the Hylids found here conditions favorable for diversification and rapid increase in numbers. [Further study of South American paleogeography induces me now (1922) to suggest the origin of the Hylidae in the southeastern Brazilian highlands when in the Cretaceous this region was isolated as an island (see p. 364).]: 6, That the genus *Protoopalina* is the oldest of the four Opalinid genera and is found in all parts of the world where climatic conditions are favorable, and has in the past been present in both Arctic and Antarctic regions whose climate now precludes the residence of Anura: 7, That the genus *Zelleriella* arose late in Patagonia and has spread a little into Australia, and that it spread into tropical America after its union with Patagonia: 8, That the genus *Cepedea* probably arose in the eastern hemisphere and migrated to America, possibly by way of Siberia and Alaska, but more probably both by this route and by a direct land-bridge between Africa and eastern Brazil: That *Cepedea* did not come to America from Africa *via* Antarctica: 9, That the genus *Opalina* is probably a polyphyletic genus and may have arisen in both the eastern and western hemispheres. [Further consideration (1922) makes me accept the genus *Opalina* as of eastern origin, but still leaves the likelihood that it is polyphyletic, *Opalina virgula* being of independent derivation from other species of the genus.]: 10, That some eastern *Opalinae* have migrated to America: 11, That one American *Opalina* has migrated by way of Alaska and Siberia to Asia and across Europe to France: 12, That the multinucleated Opalinids (*Cepedea* and *Opalina*) entered South America from the north or east and are still scarce in southern South America. [Error; *Opalina* is unknown from South America].

Metcalf (1921,*b*) gives an abstract of a second paper read before the same meeting of the American Society of Zoölogists, as follows:

A comparison of the geographical distribution of the species of Anura with that of the Anuran Opalinids gives indication of the following relations: 1, Patagonia was once connected with Australia, doubtless by way of Antarctica, and at that time climatic conditions in Antarctica were favorable for Anuran life. 2, At this time, when Patagonia and Australia were united, Patagonia was not connected with tropical America. 3, When Patagonia lost its connection with Australia it established connection with tropical America. 4, At a period later than the establishment of connection between Patagonia and tropical America, the West Indian land was still united to tropical America. 5, At a period at least as late as this there was land connection between Siberia and Alaska and a mild climate suitable for Anura. 6, For at least two geologic periods there has been, as now, a bar to the migration of moist-skinned Anura (Ranids and Leptodactylids) across northern Mexico and southwestern United States. 7, There are four lines of evidence from the Opalinids, but not conclusive, of a connection between Africa and tropical America .(not including Patagonia). This land bridge was not by way of Antarctica.

Hegner and Hsiang-Fong Wu (1921) report findings as to the relation between growth and nuclear division in *Opalina* [*larvarum*]. In young individuals with four nuclei "an increase of mass of body per nucleus, represented by an increase in area [measured from camera drawings] within the limit of 8.9 sq. mm., is the stimulus that initiates nuclear division." Division of one of the nuclei decreases the mass per nucleus by an amount represented by an area of 14.7 sq. mm., and this in spite of the fact that the body as a whole has increased in size. "There is an increase in area per nucleus up to the point where [when] one nucleus divides, then a conspicuous decrease following nuclear division, and subsequently an increase during the period when the nuclei resulting from division regain their full size, ending in a size at which the area per nucleus is approximately that present at the beginning. * * * The size of the entire specimen increases during nuclear multiplication and growth * * * but the area per nucleus remains almost constant." The data "favor the conclusion that the stimulus that initiates division acts as a rule on only one nucleus at a time, and that the division of this nucleus restores the nucleo-cytoplasmic ratio," explaining "why nuclear divisions in *Opalina* are not synchronous." "As the number of nuclei increases, their average volume and surface decreases" [an observation confirmed by my own preparations of *Opalina ranarum*].

See Appendix 1 for review of two other papers.

10. A LIST OF INSTITUTIONS IN WHICH ARE DEPOSITED SETS OF SPECIMENS OF THE SPECIES OF OPALINIDAE.

In dealing with species whose distinctive character is not very well marked, as is the case with numerous Opalinids, it is, of course, far better to have an actual specimen of the animal than to have merely a description. It is almost certain, in the case of some of the species described in this paper, that other students, with more, or better, or different material, will find it necessary to revise the taxonomy here adopted. In order to minimize so far as possible the confusion likely to result, the author has placed a set of type specimens in the United States National Museum and has asked also 16 museums and other institutions, where interesting Opalinid material is likely to be available or where protozoologists are likely to be working, to accept sets of paratypes of the new species, subspecies, and *formae* described in this paper and to allow competent students, who may so desire, to have access to them. The author retains one set. With these paratype specimens are included slides of most of the species hitherto known. The author has, however, no specimens of the following species to include in these sets: *Protoopalina acuta* (Raff), *P. dorsalis* (Raff), *P. hylarum* (Raff), *P. primordialis* (Awerinzew), *P. tenuis* (Raff), *Zelleriella binucleata* (Raff), *Z. macronucleata* (Bezzenberger), *Cepedea* (?) *flava* (Stokes), *C. lanceolata* (Bezzenberger), *Opalina cincta* (Collin), *O. coracoidea* (Bezzenberger), *O. lata* (Bezzenberger). He has but one slide each of *Cepedea virgula* (Dobell) and *Opalina* [*larvarum*] and his material of *Protoopalina saturnalis* is scant. Of a few other species there are available less than 18 slides each. Most species, however, are represented in the sets of slides distributed.

Owing to an accidental contamination of the mounting medium, not observed until nearly all the slides were made, the slides have slowly faded, until now most are practically unstained. Fortunately the fading was so slow that the author's cytological study of the material was completed before much damage was done. The work has involved making about 7,000 slides, and demounting and restaining is too forbidding a task to be undertaken, especially as the unstained slides are of value for taxonomic study.

The type series is in the United States National Museum, in Washington. The author retains a set of paratypes. In each of these two series there are included specimens of each species, subspecies, and *forma* of Opalinid from each host from which it is known and from each major region of its habitat, so far as available material is available. The other series are not quite so full. The author has also a good many extra slides of most species and some unmounted material of many species. So far as the stock left will allow, slides or material of any species will be sent (of course, without cost), upon request, to any student of this group who desires to make comparisons with his own material. Of course, extensive sets can hardly be sent, because of the expenditure of time this would involve, but requests for samples of a few species will gladly be met. Address Maynard M. Metcalf, the Orchard Laboratory, Oberlin, Ohio, United States of America.

The institutions which have accepted sets of slides are:

NORTH AMERICA.

United States National Museum, Washington, District of Columbia, United States of America.

School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland, United States of America.

American Museum of Natural History, Seventy-seventh Street and Central Park West, New York City, New York, United States of America.

Museum of Comparative Zoology, of Harvard College, Cambridge, Massachusetts, United States of America.

Department of Zoology, University of California, Berkeley, California, United States of America.

SOUTH AMERICA.

Oswaldo Cruz Institute, Rio de Janeiro (Manguinhos), Brazil.

EUROPE.

British Museum (Natural History), Cromwell Road, London, S. W., England.

Labratory of Comparative Embryology, College of France, Rue des Ecoles, Paris, France.

Zoological Labratory, Institute for Tropical Hygiene, Mauritskade 57, Amsterdam, Holland.

Zoological Museum, University of Berlin, Invalidenstrasse 43, Berlin, N. 4, Germany.

ASIA.

The Indian Museum, Calcutta, India.

Zoölogical Laboratory, The University of Tokyo, Tokyo, Japan.

Bureau of Science, Manila, Philippine Islands.

Parasitology Museum, Union Medical College, Peking, China.

Department of Agriculture and National Resources, Bureau of Science, Manila, Philippine Islands.

AUSTRALIA.

Zoological Laboratory, University of Sidney, Sidney, Australia.

AFRICA.

Wellcome Tropical Research Laboratories, Gordon Memorial College, Khartoum, Sudan, Egypt.

The South African Institute for Medical Research, Johannesburg, South Africa.

11. A LIST OF THE LITERATURE TO WHICH REFERENCE IS MADE IN THIS PAPER.

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APPENDIX I.

12. REVIEW OF TWO ADDITIONAL PAPERS UPON OPALINIDS, SUPPLEMENTARY TO SECTION 8.

Since this paper went to press there have appeared in the Archiv für Protistenkunde two papers based wholly or in part on studies of Opalinids, which are of sufficient importance to review.

Herfs (1922) studied *Opalina ranarum* among other Protozoa in regard to their ability to adapt themselves to media less dense than normal. He says that *O. ranarium* has no contractile vacuole; that it does not develop contractile vacuoles in fresh water, nor does it go to pieces very soon, but lives and appears about normal in structure and movements for a considerable time, up to 8 or 10 days may be observed; but they do die sooner than *Nyctotherus* or *Balantidium*, both of which genera live with the *Opalina* in the anuran rectum. These observations seemed to Herfs to oppose his hypothesis that the pulsating vacuole enables Protozoa to pass into unaccustomed media of less density than their normal medium without suffering injury from too great osmotic imhibition of water. This hypothesis was supported by the fact of increased activity in the pulsating vacuoles in Protozoans so transferred to less dense media and also by the appearance anew of pulsating vacuoles in marine Protozoa when transferred to fresh water, though the normal animals in sea water did not possess them. [Herfs need not abandon his hypothesis upon the basis of opposed evidence from *Opalina ranarum*, for Konsuloff (1922), in the next number of the same journal, described the system of excretory vacuoles in this *Opalina*. There seems to be no rythm of expulsion of excreta in any Opalinid, but there is occasional expulsion of both liquid and granules from the excretory pore. *O. ranarum* has numerous posterior excretory pores, according to Konsuloff.]

Konsuloff (1922) reports extensive studies upon structure, behavior, and life cycle, chiefly of *Cepedea* ["*Opalina*"] *dimidiata* and *Opalina ranarum*. In Pütter's fluid they lived up to two months, growing and dividing. Gametes taken from the recta of tadpoles passed normally through the sexual phases of the life cycle in Pütter's fluid. They live longer if finely triturated egg albumen is added to the culture medium. "*Opalina zelleri*" is but a form of *O.* [*Cepedea dimidiata*] not a distinct species (against Neresheimer

1907 and Metcalf 1909). Abnormal *Cepedea dimidiata* with slender appendages appear in starved cultures (see Neresheimer (1909) and von Linden (1909)). Valuable detailed observations are recorded as to the structure of pellicle, (Euciliate-like) ectoplasm and entoplasm. Formation of chromidia from the nuclei is described as occurring throughout life (confirming Metcalf (1909)). At no time do these give rise to nuclei, the generative nuclei being derived from the sexual nuclei by mitosis (confirming Metcalf (1909) against Neresheimer (1907)). The endosarc spherules are regarded as macronuclei because (1) they divide amitotically (confirming Tönniges (1898) against Metcalf (1909)); (2) they disappear before the completion of conjugation. [This is not the case in *Protoopalina intestinalis* or in *P. caudata* and apparently, from my preparations, is not true of *Cepedea demidiata* or *Opalina ranarum.* The endosarc spherules seem to decrease in number and may even disappear wholly from the infection cysts. They become again evident in the young Opalinids hatched from these cysts in the recta of the tadpoles. Both macrogamites and microgamites have endosare spherules in numbers proportional to the size of these animals and the same thing is true of the zygotes. Among the Euciliata the macronucleus is always a true nucleus having been derived originally from an ordinary nucleus by mitosis. The formation of nuclei from chromidia in any Protozoa is extremely improbable, the "best authenticated" instances of this process having been shown by Kofoid (1921) to be unfounded. Until the origin of endosarc spherules from normal nuclei by mitosis has been shown, the suggestion that they are macronuclei can not be entertained.]

Nutrition is by endosmosis aided by extra-cellular digestion; experimental evidence of this is offered and the digestive vacuoles opening by tubules through the pellice are described. Excretory crystals are often abundant in the endoplasm. They increase especially during the period of encystment. *Opalina ranarum* has a well-developed system of excretory vacuoles opening posteriorly by numerous pores, the middle one of which is the largest. *O. ranarum* and *C. dimidiata* are positively geotactic, and are indifferent to light and currents. They gather at the anterior end of the rectum because this is lowest. Place a frog on its back for a time and the Opalinids will be found abundantly in the posterior portion of the rectum. [? (1) In most Anura the anterior end of the rectum is the higher when the animal is sitting up; it is always so in the characteristic position at the surface of the water, and is hardly, if at all, lower than the body of the rectum when the animal is crouching; (2) the Opalinids are most numerous in the rudimentary caecum, which is the *dorsal* region of the anterior end of the rectum; (3)

Opalinids in cultures tend to gather about a bit of rectal contents or rectal wall and even when this is part way up the side of the culture dish. Chemotaxis seems a more probable explanation than geotaxis.] Interesting "Dauercysts" were found in contaminated cultures and in dead frogs; their structure and the process of their formation is described. The structure of infection cysts and the manner of their formation is described in detail, as are also endogynous infection cysts. [Doctor Fortner sent me from Michigan specimens of *Opalina obtrigonoidea* in which he had seen apparently the same sort of endogynous cysts. I have not yet studied this material carefully.]

Accumulation of much reserve nutrition is suggested as the instigating cause of the formation of infection cysts in the spring, experiments with higher temperatures and with nutrition of the host having failed to produce encystment. [?] The direct transformation of infection cysts, upon hatching in the tadpoles, into ordinary individuals which grow directly to adults without conjugation, is indicated [but not demonstrated] by observations reported. Anisogamy (confirming Metcalf (1909) against Neresheimer (1907)). Immediate encystment of zygotes (confirming Neresheimer (1907) against Metcalf (1909) and Brumpt (1915)). [This not very important matter can best be settled by study of isolation cultures.] To our conception of the life cycle of Opalinids Kousuloff adds (1) that copulation cysts [?] must [?] infect a second tadpole before they will develop to adults; (2) that some individuals hatched in the tadpoles from infection cysts develop directly to adults without conjugation. [Compare Brumpt (1915), who showed that several cycles of tadpole infection by cysts from tadpoles may occur in one Opalinid life-cycle.] Konsuloff classifies the Opalinids not as primitive Ciliates but as secondarily modified forms related to *Anoplophrya*, *Hoplitophyra*, *Discophrya*, and *Opalinopsis*, basing this judgment chiefly upon the [probably erroneous] interpretation of the endosarc spherules as macronuclei.

In an appendix Konsuloff discusses Brumpt's (1915) paper. Only one point needs mention. He supports the idea of "copulation cysts," against Brumpt's description of the cysts formed in the tadpoles as all reinfection cysts like ordinary infection cysts in adult frogs, by saying that infection cysts of *Opalina ranarum* have usually from six to eight nuclei, while "copulation cysts" have but two or after a time but one. [In my preparations of *O. ranarum* infection cysts from the adult frog show usually from 1 to 4 nuclei; but there may be as many as 12; 1 or 2 nuclei are very common.]

APPENDIX II.

13. THE REGIONAL OCCURRENCE OF THE SPECIES OF OPALINIDAE.

There was inadvertently omitted from the body of this volume any summary statement of the regional distribution of the species of Opalinidae. It seems of importance, therefore, as this volume is going to nal printing, to include such statement in a brief appendix. First will be given lists of the Opalinid species known from the major geographical regions, and there will then be added a few paragraphs of brief discussion of these lists.

I. NORTH AMERICA.

1, NORTHEASTERN NORTH AMERICA, WEST TO THE MISSISSIPPI RIVER, SOUTH TO MARYLAND.

Protoopalinae, none.
Zelleriellae, none.
Cepedeae, none.
O. *chorophili* (eastern United States, west to Texas).
O. *discophrya* (northern and eastern Canada).
O. *hylaxena* (over the whole area).
O. *hylaxena* form *orbiculata* (Massachusetts).
O. *hylaxena* form *parvinucleata* (Massachusetts).
O. *kennicotti* (northern Illinois).
O. [*larvarum*] (Nova Scotia).
O. *obtrigonoidea* (over the whole area and southwestward into the Sonoran region).
O. *obtrigonoidea americana* (Ohio, North Carolina).
O. *terrae-mariae* (Maryland).
O. *pickeringii* (District of Columbia to North Carolina, west to Ohio and the Selkirks).
O. species ?, from *Rana septentrionalis* (Ontario).

2, SOUTHEASTERN UNITED STATES, NORTH TO MARYLAND, WEST TO THE MISSISSIPPI RIVER.

Protoopalinae, none.
Zelleriella scaphiopodos (North Carolina).
Cepedea (?) *flava* (probably southeastern United States).
C. *floridensis* (Key West, Florida).
C. *obovoidea* (Florida).
Opalina carolinensis (District of Columbia, south to Florida, west to Ohio and Texas).
O. *chorophili* (eastern United States, west to Texas).
O. *hylaxena* form *georgiana* (Georgia).
O. *obtrigonoidea* (Massachusetts to Georgia and west to Arizona and Mexico).
O. *obtrigonoidea lata* (Florida, Oregon).
O. *obtrigonoidea orbiculata* (Maryland to Florida, west to Texas).
O. *pickeringii* (District of Columbia to North Carolina, west to Ohio and the Selkirks).

O. terrae-mariae (Maryland).
O. triangulata (Florida).
O. virguloidea (North Carolina, Ohio).

3, GULF COAST.

Protoopalinae, none.
Zelleriella intermedia cuneata (Brownsville, Texas).
Opalina carolinensis (District of Columbia to Florida, west to Ohio and Texas).
O. obtrigonoidea orbiculata (Maryland to Florida, west to Texas).

4, GREAT GRASSY PLAINS.

Protoopalina mitotica (Nebraska).
Cepedea cantabrigensis (Michigan, northwestward to Alaska and Great Slave Lake).
Opalina kennicotti (northern Illinois).
O. obtrigonoidea (Massachusetts to Georgia, westward to Arizona and Mexico).

5, ROCKY MOUNTAIN REGION.

Protoopalina hammondii (Wyoming to Mexico).
P. scaphiopodos (Wyoming, Colorado).
Zelleriella [of *Bufo woodhousi*] (Utah).
Opalina copei (Montana, Costa Rica).

6. SONORAN REGION—ARID STATES AND NORTHERN MEXICO.

Protoopalina mexicana (Mexico).
P. ovoidea (Texas).
Zelleriella couchii (Texas).
Z. hirsuta (Arizona).
Z. intermedia (Mexico).
Z. intermedia cuneata (Brownsville, Texas).
Cepedea mexicana (Matamoras, Mexico).
C. species ?, from *Hyla versicolor chrysoscelis* (New Braunfels, Texas).
Opalina discophrya (New Mexico).
O. gigantea (Texas).
O. oblanceolata (Arizona, northern Mexico).
O. obtrigonoidea (Massachusetts to Georgia, west to Arizona and Mexico).
O. spiralis (Texas, Arizona).
O. virguloidea (Mexico).
O. woodhousi (Arizona, Utah).
O. species ?, from *Hyla versicolor chrysoscelis* (New Braunfels, Texas).

7, PACIFIC COAST.

Protoopalinae, none.
Zelleriella ranaxena (southern California).
Z. [*of Bufo punctatus*] (*southern California*).
Cepedeae, none.
Opalina draytonii (California, Lower California).
O. obtrigonoidea maxima (San Francisco, western Canada, Alaska).
O. obtrigonoidea lata (Oregon, Florida).
O. oregonensis (Oregon, Vancouver Island).
O. virguloidea (San Francisco, south into Mexico, also Ohio to North Carolina).

II. CENTRAL AMERICA AND WEST INDIES.

1. TROPICAL CENTRAL AMERICA.

Protoopalina xyster (Tehauntepec, Mexico).
Zelleriella [*atelopodos*] (Costa Rica).
Z. atelopyxena (Costa Rica).
Z. [of *Bufo strenosignatus*] (Tehuantepec, Mexico).
Z. bufoxena (Nicaragua).
Z. dendrobatidis (Costa Rica, Nicaragua).
Z. [*engystomopsis*] (Taboga Island, Panama).
Z. hypopacheos (Guatemala).
Z. leptodactyli (Tehuantepec, Mexico: Guatemala).
Z. opisthocarya (Nicaragua, Costa Rica).
Z. opisthocarya [of *Bufo monxiæ*] (Guanajuato, Mexico; Yucatan).
Z. opisthocarya [of *Bufo typhonius*] (Panama).
Cepedea baudinii (Cordova, Mexico; Guatemala).
C. dolichosoma (Nicaragua, Costa Rica).
C. globosa (Costa Rica).
C. occidentalis (Nicaragua).
Opalina copei (Costa Rica, Montana).
O. guatemalae (Guatemala).
O. helenae (Nicaragua, Costa Rica).
O. helenae phyllomedusae (Mexico).
O. moreletei (Guatemala).
O. obtrigonoidea (Guanajuato, Mexico, extends northeastward to Massachusetts).
O. obtrigonoidea austricola (Costa Rica; Guatemala; Tabasco, Mexico).
O. panamensis (Panama).
O. species ?, from *Bufo haematiticus* (Nicaragua).

2. WEST INDIES, INCLUDING BAHAMA ISLANDS.

Protoopalina bufonis (Cuba).
Zelleriella leptodactyli (Porto Rico, Mexico).
Z. microcarya (Porto Rico).
Z. [of *Hyla septentrionalis*] (Bahama Islands).
Z. species ?, from *Bufo peltocephalus* (Cuba).
Cepedeae, none.
Opalinae, none.

III. SOUTH AMERICA.

1. NORTHERN AND NORTHWESTERN SOUTH AMERICA, INCLUDING THE GUIANAS, VENEZUELA, COLUMBIA, ECUADOR, PERU, AND NORTHWESTERN BRAZIL.

Protoopalinae longinucleata (Ecuador).
Zelleriella binghami (Peru).
Z. [*boulengeri*] (Columbia).
Z. cusconis (Peruvian Andes).
Z. magna (Venezuela).
Z. [of *Bufo spinulosus*] (La Paz, Brazil; Lake Titicaca, Peru).
Z. paludicolae (Tolcahuano, Chile).
Z. telmatobii (Ecuador; Blanca Island, Peru).
Z. [*trinitatis*] (Venezuela).
Z. venezuelae (Venezuela).
Cepedeae, none.
Opalinae, none.

2, EASTERN BRAZIL.

Protoopalinae, none.
Zelleriella brasiliensis (Rio de Janeiro, Brazil; La Plata, Argentina).
Cepedea multiformis (Bonito, Brazil; Nicaragua).
Opalinae, none.

3, AMAZON VALLEY.

Opalinidae unknown.

4, SOUTHERN SOUTH AMERICA, INCLUDING PARAGUAY, URUGUAY, ARGENTINA PATAGONIA, AND CHILE.

Protoopalina diplocarya (Patagonia).
P. rhinodermatos (Chile).
Zelleriella antilliensis (?) (Uruguay).
Z. brasiliensis (La Plata, Argentina; Rio de Janeiro, Brazil).
Z. darwinii (Chile).
Z. hylaxena (Paraguay).
Z. patagoniensis (Straits of Magellan, Patagonia).
Cepedea dimidiata [*paraguensis*] (Paraguay).
Opalinae, none.

IV. PALAEARCTICA.

1, ASIA NORTH OF HIMALAYAN MOUNTAINS.

Protoopalina axonucleata (Korea, Manchuria).
P. axonucleata lata (China).
P. macrocaudata (Korea, Manchuria).
P. orientalis (Korea, Manchuria).
Zelleriellae, none.
Cepedea buergeri (Japan).
C. buergeri sinensis (Hong Kong, China).
C. dimidiata orientalis (Japan).
C. formosae (Hong Kong, China; Formosa).
C. fujiensis (Japan).
C. longa (Japan, Formosa).
C. multiformis [of *Polypedates schlegelii*] (Japan).
C. pulchra japonica (Japan).
Opalina asiatica (Shanghai, China).
O. [*bufoxena*] (Manchuria).
O. japonica (Japan).
O. obtrigona (Manchuria, Jerusalem).
O. raddei (China).

2, EUROPE AND NORTHERN AFRICA.

Protoopalina caudata (Europe).
P. caudata discoglossi (Sardinia, Algeria).
P. intestinalis (Europe, northern Africa).
P. pelobatidis (Austria).
Zelleriellae, none.
Cepedea dimidiata (Europe).
C. dimidiata form *zelleri* (Europe).
C. hispanica (Spain).
C. minor (France).
C. saharana (Algiers).
Opalina cincta (France).

O. ranarum (Europe, northern Africa ?).
O. ranarum form *arvalis* (lower "Austria").
O. ranarum form *cinctoidea* (Germany).
O. ranarum form *lata* (Italy).
O. ranarum form *parvipalmatae* (France).
O. ranarum form *truncata* (Germany).

V. SOUTHERN AND SOUTHEASTERN ASIA[44] AND MALAYSIA.

Protoopalina filiformis (Formosa, Java, Billeton Island near Sumatra).
P. formosae (Formosa).
P. montana (Java).
P. quadrinucleata (Java).
Zelleriellae, none.
Cepedea borneonensis (Borneo).
C. formosae (Hong Kong, China: Formosa).
C. lanceolata ("Asia").
C. longa ("Asia", Japan, Formosa).
C. ophis (Formosa, Billeton Island near Sumatra).
C. pulchra (Cochin China).
C. pulchra japonica (Java).
C. segmentata (Cochin China, Sumatra, Java).
C. spinifera (Java).
Opalina rotunda (Siamese Cambodia).

VI. AUSTRALASIA.

1, PAPUASIA.

Protoopalina papuensis (Papua).
Zelleriellae, none.
Cepedeae, none.
Opalinae, none.

2. AUSTRALIA, TASMANIA.

Protoopalina acuta (Australia).
P. adelaidensis (Australia).
P. australis (Australia).
P. dorsalis (Australia).
P. hylarum (Australia).
P. intestinalis ? (Australia).
P. peronii (Australia).
P. tenuis (Australia).
Zelleriella binucleata (Australia).
Cepedeae, none.
Opalinae, none.

VII. AFRICA, MADAGASCAR, INDIAN OCEAN ISLANDS.

1, TROPICAL AND SOUTHERN AFRICA.

Protoopalina africana (The Cameroons).
P. mossambicensis (Mozambique).
P. nutti ("British East Africa").
P. primordialis ("German East Africa").

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[44] Unfortunately very few Indian Anura have been searched for Opalinidae.

P. regularis (Gold Coast, "British East Africa").
P. stevensoni (Sudan).
P. xenopodos (Belgian Congo).
Zelleriellae, none.
Cepedeae madagascariensis [of *Hyperolius*] (West Africa).
C. magna (The Cameroons).
C. phrynomantidis ("British East Africa").
Opalina camerunensis (The Cameroons).
O. natalensis (Sudan).
O. species ?, from *Rana mascareniensis* (Gold Coast).

2, MADAGASCAR, SEYCHELLES ISLANDS, CEYLON.

Protoopalinae, none.
Zelleriellae, none.
Cepedea madagascariensis (Madagascar).
C. seychellensis (Seychelles Islands).
Opalina virgula (Ceylon).

From these lists we see that northeastern North America has Opalinids only of the genus *Opalina*, and that the same is true of southeastern United States except for probably a single species each of the genera *Zelleriella* and *Cepedea*. *Zelleriella scaphiopodos* of North Carolina is probably an immigrant from Central America by way of the Gulf coast. *Cepedea floridensis* from Key West, Florida, doubtless also came from Central America, either by way of the Gulf coast or from the Greater Antilles. The southern tip of Florida shows many close faunal affinities with the West Indies and Central America. The eastern North American *Opalinae* all belong to the *Opalinae angustae.*

The region of the north coast of the Gulf of Mexico is not distinct in its Opalinid fauna. Its sole *Zelleriella* doubtless came from Central America. Its two *Opalinae* are forms with chiefly eastern distribution.

The great grassy plains also are not well demarcated in their Opalinid fauna. Their only *Protoopalina* belongs to the exclusively North American subgenus resident in *Scaphiopus*, this particular species (*mitotica*), however, having adopted the Urodele host *Ambystoma tigrinum.* The *Opalinae* are of the characteristic American subgenus *Opalinae angustae* and two of them are of very wide range, one being spread from Massachusetts to Mexico and the other ranging to the north and west to the regions of such extreme cold that Anura can spread no further.

The Rocky Mountain region gives us two *Protoopalinae* of the subgeneric group characteristic of *Scaphiopus*, and a single *Zelleriella* from Utah which might about as well be assigned to the Sonoran region. It is evidently an immigrant from the south. There is also one *Opalina*, found also in Costa Rica.

The Sonoran region, comprising the semidesert territory of southwestern United States and northern Mexico, has representatives of all genera of Opalinidae. *Protoopalina mexicana* belongs to the group of species characteristic of *Scaphiopus* and they were apparently developed in North America from an ancestor immigrant from Asia by way of Alaska. The other *Protoopalina* is a southern form intermediate between the genera *Protoopalina* and *Zelleriella.* The four Sonoran *Zelleriellae* are, of course, immigrants from further south. Of the two Sonoran *Cepedeae* one is of uncertain affinities; the other, from a Texan *Hyla*, seems to belong to a group containing eastern Asian and Malaysian species, a group not represented in northern Asia. The *Opalinae* are chiefly Western Hemisphere narrow species, though we find in *O. gigantea* an immigrant representative of the Eastern Hemisphere broad species.

On the Pacific coast we find no *Protoopalinae.* Well to the south are two *Zelleriellae*, evidently immigrants from further south. No *Cepedeae* are present. The *Opalinae* are narrow species except for *O. draytonii*, a broad form immigrant from eastern Asia.

From tropical Central America we know but a single *Protoopalina*, *xyster*, one of the two species which show transition toward the genus *Zelleriella.* The *Zelleriellae* are abundant, eleven species being known, tropical America being the present major habitat of the *Zelleriellae.* Of the four *Cepedeae* two (*globosa* and *baudinii*) show African affinities and are probably derived from ancestors immigrant from Africa by way of the Guianas, while the two other species belong, one to a group with mostly Asia-Malaysian species, and the other to a group represented both in Asia-Malaysia and in the Seychelles Islands, the latter occurrence being a puzzle and requiring further study. The *Opalinae* are narrow forms, except one which is broad and two which intergrade between the narrow and broad groups.

The West Indian species show tropical continental American affinities. The *Protoopalina*, *bufonis*, seems to belong to a group with both African and South American representatives and which we regarded as having arisen in South Atlantis. The four *Zelleriellae* are, of course, tropical American. No *Cepedeae* nor *Opalinae* are known from the West Indies.

Northern and northwestern South America shows one *Protoopalina* and nine *Zelleriellae.*

From eastern Brazil we know no *Protoopalina* and of course no *Opalina.* There is one *Zelleriella*, found also further south, in Argentina. The only known *Cepedea* is found in Central America and we have regarded it as belonging to a group with chiefly Asia-Malaysian species, though the same group shows apparently one representative in the Seychelles Islands and possible one in Spain. This group is but a loosely affiliated one showing a rather wide range of intergrades from the *dimidiata*-like forms toward the *longa* forms.

Unfortunately no Opalinids are known from the great Amazon Valley.

In southern South America we find all genera, except, of course, *Opalina* which is not found in America south of Panama. Of the two *Protoopalinae*, one, *diplocarya*, is a relict of the most archaic Equatorian group of species, and the other belongs to a group with African affinities. It is interesting to find six *Zelleriellae* here in the southern part of the continent, though they have but a single known representative in eastern Brazil. *Zelleriellae* are abundant in Central America, in northern and northwestern South America and in southern South America, but not, so far as our data go, in central South America. The Paraguayan *Cepedea* is a serious puzzle, for it so closely resembles the Euro-Asian *Cepedea dimidiata* as to be indistinguishable. We have called it a distinct subspecies only because of its so distant habitat.

The Eastern Hemisphere is sharply demarcated from the Western Hemisphere by the absence of *Zelleriella*, except one species in Australia and barely possibly another in "Asia," also by the absence of narrow *Opalinae*, except one immigrant species from North America and *O. virgula*, of doubtful affinities.

Palaearctica, in its Asian portion, shows four *Protoopalinae*, two of them similar to European species, eight *Cepedeae*, two of which show relationships to Malaysian forms, and six *Opalinae* all broad except for one immigrant from America. Ceylon is not included.

Europe and northern Africa show four *Protoopalinae* representing two ancient groups, five *Cepedeae* similar to northern Asian forms, and two or three species of *Opalinae*, one the narrow immigrant from America, the others *O. ranarum* and the somewhat similar *O. cincta* which might perhaps be regarded as a subspecies of *ranarum*. The broad *Opalinae* are therefore dominantly Asian rather than European.

Southern and southeastern Asia and Malaysia give us four *Protoopalinae* of groups more modified than those in Europe, eight *Cepedeae*, including the most archaic and the most modified species, also a single broad *Opalina*.

Among the Australasian Opalinids we find in Papua only a single very archaic *Protoopalina*. In Australia we find another species of this most archaic group in the genus *Protoopalina* and seven other species, all of little modified character except one (*tenuis*), which shows close similarity to the elongated slender Malaysian *P. filiformis*. There is known a single Australian species of *Zelleriella* closely similar to the American forms. No multinucleate Opalinids are in Australia.

From tropical and southern Africa we know seven *Protoopalinae* of wide variety—one (*xenopodos*) belonging to the most archaic group of all and one other (*africana*) approaching in form the very slender

Malaysian and Australian species. The three *Cepedeae* all belong to one subgeneric group confined to Africa and Madagascar except for one species in Florida, which seems related, though not very closely so. The three *Opalinae*, all of course broad, are probably comparatively recent immigrants from Euro-Asia and show little of special interest.

Two *Cepedeae* and one *Opalina* are known from the islands which are remnants of formerly more extensive Indian Ocean lands uniting southern Africa with India. Madagascar and the Seychelles Islands each furnish a single species of *Cepedea*, the two very similar and very closely related to a western African species. Ceylon has a strange *Opalina* of narrow form, but probably not related to the *Opalinae angustae* of North America.

The general indications from this regional survey are in agreement with the conclusions and suggestions already made in the body of this volume, except that the greater abundance of the species of *Zelleriella* in Central America (11), the Antilles (4), and northern South America (9) than in southern South America (6) seems to cast doubt upon the origin of this genus in Argentina-Patagonia, as we have proposed. The Leptodactylidae, which are doubtless the original hosts of the Zelleriellas, show about an equal number of species in the two contrasted regions, thought the area of the more northern region is many times the greater. There are also in the northern region a far greater variety of hosts for *Zelleriella* to adopt. On the whole there seems no sufficient ground for changing our suggestion of a southern origin for the Leptodactylidae and their *Zelleriella* parasites.

A few words further may well be added as to the origin of the Hylidae. We have suggested that this family arose in the highlands of eastern Brazil, north of the sea which separated Patagonia-Argentina from tropical America. The Hylidae and Leptodactylidae seem very closely related, and the two families probably arose in adjacent regions and from common ancestors. If the Leptodactylidae arose in southern South America, then the Brazilian highlands is the most probable place of origin for the Hylidae. Again, if the Hylidae had arisen further north, in any region connected with the eastern Pacific land strip while this was complete from Ecuador to Asia, it seems inexplicable that the Hylidae should not have extensively colonized Asia as they have North America. It seems that the South American home of the Hylidae was united to Australasia, but not to Asia, since the time of the origin of the Hylids, except for the comparatively modern connection between South America and Siberia by way of the Isthmus of Panama and North America.

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